The search for biomarkers in the growth prediction of the West Coast Rock Lobster, *Jasus lalandii*

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Notes

This thesis is presented in the format prescribed by the Department of Animal Sciences, Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by a summary chapter with the study objectives, followed by a general introduction chapter and culminating with a chapter for elaborating a general discussion and conclusions. Referencing format used is in accordance with the requirements of the Journal of Experimental Marine Biology and Ecology. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Results of this thesis have been published in the following journals:

Munian, A., Cockcroft, A., Hoffman, L.C., Auerswald, L., 2021. Depot lipids in mature palinurid decapods. Invertebr. Reprod. Dev. 65, 1-11. doi.org/10.1080/07924259.2020.1846631

Munian, A., Cockcroft, A., Hoffman, L., Auerswald, L., 2021. Analysis of biological and biochemical parameters of adult male spiny lobsters *Jasus lalandii* for identification of possible growth predictors. Fisheries Res. 243, doi.org/10.1016/j.fishres.2021.106061.

Summary

The West Coast rock lobster (WCRL) *Jasus lalandii* is a key ecological species and an important fisheries resource in South Africa. The resource is heavily depleted to 1.8% of pristine levels. The fishery is managed according to an operational management procedure to sustainably rebuild the stock, using an annual catch limit and effort control, determined from Catch per Unit Effort, fisheries independent abundance index and growth data. Since these derive from the preceding season, the present research aimed to identify timelier predictors for the next season.

The first part of this study analysed biological parameters of male and female WCRL of male commercial size over two years to determine reserve accumulation during two growth (moult) and reproductive (ovarian) cycles. A historically fast-growth- and a slow-growth area were compared - in relation to their annual male somatic growth rate. Moreover, moult stage and biochemistry of hepatopancreas and ovaries were investigated from the fast growth area. The second part specifically investigated reserve accumulation in the high-growth area with a focus on lipid reserves.

The study confirmed that annual growth increment in Hout Bay is still about 1 mm higher than at Olifantsbos and inter-annual differences persist within areas. Growth remains at the low level that continues since 1987, indicating that factors for slow growth persist. Results also confirmed seasonal reserve accumulation in male hepatopancreas in preparation for moult/growth in both areas, whereas there was no such seasonality in female hepatopancreas. Concomitant with male hepatopancreas size, female gonad size increased from a minimum in winter to a maximum shortly before spawning. Reserve accumulation varied inter-annually and in extent and timing for both genders. Lipids and proteins accumulated during the moult cycle in male hepatopancreas and in ovaries but not in female hepatopancreas. During the ovarian cycle, reserves accumulated in the ovaries but not in the female hepatopancreas. Some indications of an involvement of the female abdominal muscle in reproduction (ovary maturation) were revealed: It is up to 8% of body size larger than in males and its size declines as ovary cycle progresses.

During ovary maturation, females from the Hout Bay area had 42 – 120% more lipids available (total in hepatopancreas and ovaries) than males, meaning that a) females have a higher food consumption rate, b) the area provides sufficient food to support both growth and reproduction, and c) there is surplus food available for males to support optimal somatic growth. In

conclusion, females do not need to (completely) prioritise partition of reserve between growth (ovary) and reproduction (hepatopancreas) in this area. The fatty acid profile of male and female hepatopancreas and female gonads did not change during accumulation and no changes in fatty acid profiles occurred during both moult- and reproductive cycles. Presumably, the quality of the available food in the fishing area is unchanged during reserve accumulation and optimal for growth and reproduction.

The project aimed at the identification of potential predictors for male somatic growth as an input for annual catch determination. Based on the prerequisite that a potential predictor must display distinct seasonal variation, several parameters were investigated. However, many of them, such as moult stage and fatty acid profile were found unsuitable. Only three sets of data emerged that have the potential to serve as future predictors: 1) lipid accumulation for growth in the male hepatopancreas, 2) reserve accumulation for reproduction in the female gonads and 3) the difference between male and female lobsters in total available lipids. Only 1) correlated well with growth increment during subsequent moult in the area. However, 2) and 3) have strong potential but require additional research.

Although these results are not yet robust enough to qualify for the use in stock assessment, they provide important initial information for the development of a predictor for male growth increment in the subsequent moult. The project also produced important information to design a future research strategy to answer remaining questions and provide further advice for fisheries management of the WCRL resource.

Opsomming

Die Weskus rotskreef (WCRL), *Jasus lalandii*, is ‘n ekologies sowel as ekonomies belangrike kreefspesie in Suid-Afrika. Huidige getalle van die spesie is slegs sowat 1.8% van die vlakke wat verwag sou word in ongerepte toestande. Die verwydering van die kreefspesie word tans streng bestuur om in ‘n volhoubare poging om getalle van die spesie te laat toeneem. Aangesien jaarlikse limiete bereken word deur gebruik te maak van die vorige jaar se data, is die doel van hierdie navorsing om akkurate voorspellings vir die volgende seisoen te vergemaklik.

In die eerste gedeelte van die studie is die biologiese parameters van manlike sowel as wyfie krewe met kommersiële grootte bo 2 jaar ouderdom gebruik om reserwe opeenhoping gedurende twee groei -(dop verwerp) en voortplantingsiklusse te bepaal. Data vanaf histories vinnig sowel as stadige groei areas is vergelyk in verhouding met die jaarlikse manlike somatiese groei tempo. Die groei (dop verwerp) sowel as biochemie van die hepatopankreas en eierstokke in die area met vinnige groei is bestudeer. In die tweede deel van die studie, is spesifiek gefokus op reserwe lipied akkumulasie in die area met vinnige groei.

Die studie het bevind dat die jaarlikse toename in die Houtbaai area ongeveer 1 mm hoër is as in die Olifantsbos area. Jaar tot jaar verskille volhard in die verskillende areas. Die lae groeivlakke soos waargeneem sedert 1987 duur voort wat aandui dat die faktore wat ‘n lae groeitempo veroorsaak, nog steeds teenwoordig is. Resultate bevestig ook seisoenale reserwe opeenhoping in manlike hepatopankreas ter voorbereiding vir die groei (dop verwerp) fase. In teenstelling hiermee, is geen seisoenale opeenhoping in die vroulike hepatopankreas waargeneem nie. Jaar tot jaar variasie in die omvang sowel as tydsintervalle van reserwe opeenhoping is in beide geslagte waargeneem. Gedurende die groeifase, is die opeenhoping van lipiede sowel as proteïene in manlike hepatopankreas sowel as vroulike eierstokke waargeneem. Geen opeenhoping is waargeneem in vroulike hepatopankreas nie. Gedurende die ovariale siklus vind reserwe opeenhoping plaas in die eierstokke, maar nie in die vroulike hepatopankreas nie. ‘n Mate van betrokkenheid van die vroulike abdominale spier is waargeneem in voortplanting (rypwording van die eierstokke). Die vroulike abdominale spier is tot 8% groter as by die manlike kreef, en die grootte neem af soos wat die ovariumsiklus vorder.

Tydens die ovariumsiklus was die lipiedvlakke by vroulike krewe in die Houtbaai area (totaal in hepatopankreas sowel as eierstokke), 42 – 120% hoër as by manlike krewe. Die hoër vlakke by die vroulike krewe, beteken, a) hoër tempo van voedselopname, b) genoegsame nutriënte in

die area om groei sowel as voortplanting te ondersteun, c) surplus voeding vir manlike krewe beskikbaar om optimale somatiese groei te bevorder. Ter afsluiting, blyk dit dat daar minimale noodsaaklikheid is vir die partisie van reserwes tussen ovarium en hepatopankreas by wyfie krewe in die area. Daar was geen verandering in die vetsuurprofiele van mannetjie en wyfie hepatopankreas sowel as eierstokke van die wyfies tydens groei- en voortplantingsiklusse. Die kwaliteit van beskikbare voeding in die area, is waarskynlik onveranderd tydens die opeenhoping van reserwes en is optimaal vir groei en voortplanting.

Die doel van die projek was die identifikasie van potensiële voorspellingsmeganismes vir somatiese groei by mannetjie krewe as inset vir die bepaling van jaarlikse vangs limiete. Die voorvereiste vir ‘n potensiële voorspellingsmeganisme is dat daar duidelike seisoenale variasies moet voorkom en daarom is verskeie parameters tydens die studie ondersoek. Daar is egter bevind dat verskeie parameters, soos die groeifase (vervel) sowel as vetsuurprofiele nutteloos was. Drie stelle data is bevind as potensiële toekomstige voorpellingsmeganismes: 1) opeenhoping van lipiede vir groei in manlike hepatopankreas, 2) reserwe opeenhoping vir voortplanting in vroulike geslagskliere en 3) die verskil tussen mannetjie en wyfie krewe in totale beskikbare lipied vlakke. Slegs 1) korreleer goed met ‘n toename in groei gedurende die daaropvolgende dop verwerp fase in die area. Punte 2) en 3) toon goeie potensiaal maar verdere navorsing is nodig.

Alhoewel die studie resultate nie robuus genoeg is om gebruik te word vir die voorspelling van getalle nie, is daar voldoende aanvanklike inligting vir die ontwikkeling van ‘n voorspellingsmeganisme vir ‘n toename in groei van die manlike spesie. Die resultate van die projek voorsien ook belangrike inligting vir toekomstige navorsing rakende die bestuur van die Weskus Rotskreef hulpbron.

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General Discussion

Addenda

Chapter 1

General introduction and project aims

Globally, marine crustacean resources support invaluable fisheries and aquaculture (FAO, 2020). The commercially most important taxa are all decapods, namely clawed-, spiny-, and slipper lobsters, crabs and shrimps/prawns (Briones-Fourzán and Lozano-Àlvarez, 2015). They include 248 lobster species (Chan, 2010). The global importance of lobsters is highlighted by landings of the capture fisheries of more than 300000 t in 2018 (FAO, 2020) of which palinurids (spiny or rock lobsters) contribute 28% (Penn et al., 2019) at a commercial value of more than US$ 4 billion (Radhakrishnan et al. 2019). In their respective habitats, these species also play an essential role in ensuring ecosystem diversity (Barkai and Branch, 1988; Briones-Fourzán and Lozano- Àlvarez, 2015).

In South Africa, two of the more than 20 commercial fisheries are spiny lobster fisheries. These are the fishery for West Coast- (*Jasus lalandii*) and South Coast- (*Palinurus gilchristi*) rock lobsters (DEFF, 2020). The fishery for West Coast rock lobster (WCRL) is the most important lobster fishery with an annual value of more than 500 million rand (~US $40 m), providing approximately 4,200 jobs (DEFF, 2020). The resource is heavily depleted and fishing pressure, including poaching, is heavy (DEFF, 2020). The harvestable component is currently estimated at 1.8% of pristine (i.e. pre-fishing) levels (DEFF, 2020).

The commercial fishing area for WCRL is situated in the Benguela Current Large Marine Ecosystem (BCLME; Pollock, 1986), one of the biggest Eastern boundary upwelling systems (Summerhayes et al., 1995). By nature, it is very variable in the short- to medium term. The WCRL is therefore exposed to a variety of environmental changes. Due to upwelling, water parameters are continually changing over the short term (Bailey and Chapman, 1991; Hutchings et al., 2009; Summerhayes et al., 1995). This seasonal upwelling also leads to algal blooms which subsequently collapse and cause periods of acute hypercapnic hypoxia (Pitcher and Probyn, 2010). These short-term changes, however, may become more prolonged as anthropogenic CO_2 concentrations continue to increase at historically unprecedented rates, greater than the geological system can counter (Blackford and Gilbert, 2007).

It is possible that the WCRL population has already reacted to such (currently unknown) environmental change by a behavioural response - a southward shift of abundance (Cockcroft et al., 2008). Physiologically, *J. lalandii* is well adapted to master challenges from ocean

acidification and warming (Knapp et al. 2015, 2016, 2019). However, the impact of hypoxic periods is severe (Cockcroft, 2001). In addition, physiological limits may be reached in future and, in the long-term, stressors related to a reduced seawater pH may contribute to a decreased somatic growth rate in the WCRL due to the impact on exoskeleton calcification (Kleypas and Langdon, 2000) and the cost of metabolic adjustments such as acid-base regulation (Pörtner et al., 2004).

Similar to other palinurids, the life cycle of *J. lalandii* is complex, especially its larval phase (Booth, 2006). After subsequent settlement, juvenile lobsters moult often and grow fast (Dubber et al., 2004; Hazell et al., 2001). After reaching sexual maturity, moult- and reproductive cycles of male and female WCRL become closely synchronised and annual. Males moult in late spring to early summer, well before females in late austral autumn to early winter and prior to the mating period in winter (Heydorn, 1969; Newman and Pollock, 1974). This means that metabolic reserves in the male hepatopancreas and female gonads build up during the same period and experience environmental influences simultaneously (Heydorn, 1969; Cockcroft, 1997). Consequently, a positive relationship between male growth and female fecundity exists (Melville-Smith et al., 1995).

The current system of management of the West Coast rock lobster fishery has evolved over several decades and currently follows an Operational Management Plan (OMP) aimed at ensuring sustainable management of the resource. This includes the setting of annual Total Allowable Catches (TAC), an effort control component, a defined fishing season and fishing areas, a minimum legal size of males only and a ban on the retention of females (Cockcroft and Payne, 1999). The input for annual TAC setting is currently derived from commercial Catch Per Unit Effort (CPUE) of both hoop net (row boats and deck boats) and trap fisheries, a Fisheries Independent Monitoring Survey (FIMS) and area-specific tagging of male sized lobsters prior to the start of the fishing season (DEFF, 2020). From these activities, recent male somatic growth rate and abundance are main factors that feed into the OMP and determine the TAC (Johnston et al., 2012).

Currently, these TAC inputs pre-date the fishing season and may not reflect the state of the resource at the beginning of the new season. They only indirectly consider the physiological condition of lobsters and are unable to predict the male somatic growth rate. This is suboptimal for sustainable management of the resource. The present dissertation is therefore an attempt to search for reliable physiological and biological data that can be used for predicting population-

and resource development and help to ensure sustainable management. Due to the above-mentioned synchronisation of biological cycles, such predictors may be found in males and females.

The overall aims/objectives of the study were therefore:

- To analyse somatic growth rates of male WCRL of commercial size at a high growth- and a low growth fishing area and compare them with historical data.

- To determine and compare the seasonal variation in the biological cycles (moult and somatic growth rate) of male lobsters and in the moult- and ovary cycles of female lobsters from the two areas.

- To analyse biochemical composition of male and female hepatopancreas and female gonads from lobsters of the fast growth area during this period to obtain details on reserve accumulation for male- and female growth (hepatopancreas) and reproduction (ovaries) and reserve partitioning between growth and reproduction in females.

- To analyse changes in storage organ parameters as well as lipid composition during the parallel accumulation phases of male and female hepatopancreas and female gonads from the fast growth area.

- To analyse above data from males and females for suitability and reliability as physiological predictors for male somatic growth and possibly provide timelier physiological recommendations for inclusion of such data into the OMP.

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Chapter 2

Literature Review

Biochemical indicators for growth and reproduction in the West Coast rock lobster *Jasus lalandii*

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Refer to Addendum A

Abstract

Decapods form the backbone of global crustacean fisheries and aquaculture. Palinurids contribute a small percentage of global decapod catches but are of high commercial value. They are currently rarely cultured, but some fast-growing tropical species are regarded as one of the "most promising emerging species for aquaculture". Recent advances in palinurid aquaculture have raised interest in their metabolism, including that of depot lipids. Probably due to past difficulties in palinurids' aquaculture, knowledge on lipid metabolism of adults is poor, especially when compared with commercially important aquacultural decapods. However, interpolation of data from other decapod infraorders such as penaeids, from which the bulk of knowledge on crustacean lipids is derived, is problematic. Palinurids have a completely different life cycle, demanding different metabolic requirements than related decapod taxa. Providing energy during moulting and gonad development, depot lipids play a central role in growth and reproduction of palinurids. The high relevance of lipid digestion and accumulation is indicated by the high hepatopancreas lipid content (higher than in other decapod taxa) and the increasing importance of lipids with ontogenesis. The present review intends to provide an overview over the currently available information on the role of those depot lipids in the biology of mature palinurid decapods and regulation of their metabolism.

Keywords: palinurid, depot lipid, growth, reproduction, metabolism, life cycle

2.1 Introduction

Decapod crustaceans form the backbone of global crustacean fisheries and aquaculture (FAO, 2020). Palinurids (spiny or rock lobsters) contribute 28% of the 301 000 tons of total catches of all lobster groups (Penn et al., 2019) with a commercial value of more than US$ 4 billion $ (Radhakrishnan et al., 2019). In South Africa, the fishery of the West Coast rock lobster (WCRL), *Jasus lalandii,* is one of the most important due to its high value (2016 = R 538 m; ~$40 m) of which 98 % is exported (Fishing Industry Handbook, 2018). The fishery is also an important provider of employment for about 4 200 people, most of them along the South African West coast where impoverished communities live (DEFF, 2020). The management of the fishery has developed over several decades and currently follows an Operational Management Plan (OMP) to achieve sustainable management of the resource by annual Total Allowable Catches (TAC), an effort control component, defined fishing season and fishing areas, a minimum legal size and a ban on the retention of berried females (Cockcroft and Payne, 1999). Inputs for annual setting of the TAC are currently derived from commercial Catch Per Unit Effort (CPUE) of both hoop net (row boats and deck boats) and trap fisheries, Fisheries Independent Monitoring Survey (FIMS), and recent somatic growth rates observed by area-specific tagging of male sized lobsters prior to the start of the fishing season (DEFF 2020). In addition to fishing pressure, environmental influences have impacted the resource in recent decades including reduced growth rates (Pollock et al., 1997), low-oxygen events that caused mass stranding of lobsters (Cockcroft, 2001) and an eastward shift in lobster distribution (Cockcroft et al., 2008). The current TAC inputs pre-date the new fishing season and may not reflect the state of the resource at the beginning of the new season. They also only indirectly consider the physiological condition of lobsters. In addition, the current approach does not allow for the prediction of male somatic growth rate. A reliable physiological index, or predictor, would therefore be a valuable improvement to the TAC setting process. This would be novel not only in South African fisheries but also worldwide where the use of physiological indicators as input data for setting catch levels is scarce.

2.2 General biology of the West Coast rock lobster

The palinurid decapod *J. lalandii* is a slow-growing, cold- to temperate water palinurid species and is probably the best studied crustacean in South Africa. It lives mainly on rocky reefs in depths ranging from the intertidal to 200 meters along a stretch of coastline that spans from Walvis Bay (Namibia) in the northern Benguela Current to East London on the east coast of South Africa (Groeneveld et al., 2010). The species is a benthic predator and feeds

predominantly on sea urchins and various species of available mussels (Barkai and Branch, 1988; Barkai et al., 1996; Booth, 2006). However, feeding preferences are adaptable in different areas to abundance and availability of prey species (Griffiths and Seiderer, 1980).

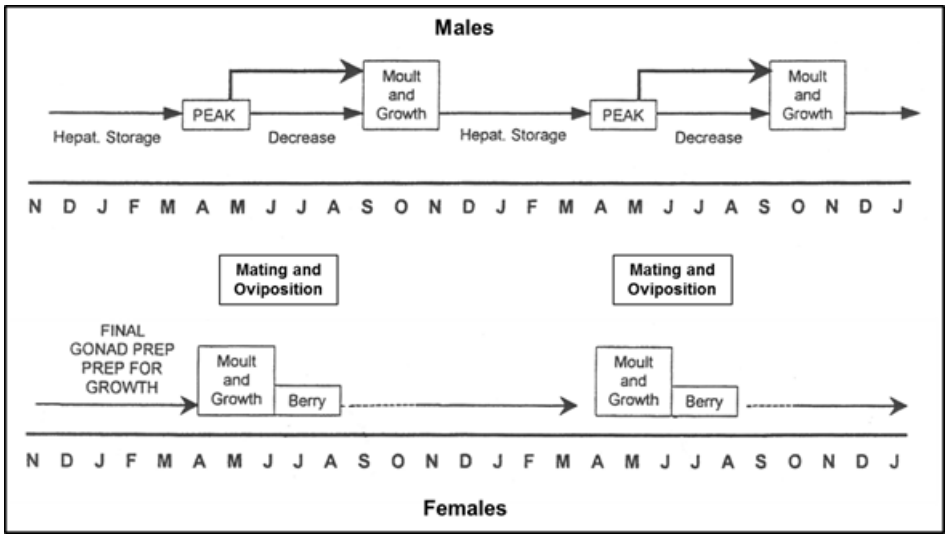

Figure 1. Synchronisation of seasonal events between growth- and reproductive cycles of male and female *J. lalandii*. Reproduced with permission of A.C. Cockcroft (DFFE).

The life cycle of *J. lalandii* is complex (Booth, 2006). Moult- and reproductive cycles of males and females are seasonal and tightly coupled. Mating and spawning occurs during austral autumn/winter between June and July (Figure 1), with external fertilization of eggs (Booth, 2006). At the time of oviposition and spawning, the female exoskeleton is still relatively soft (Heydorn, 1965; 1969; Berry and Heydorn, 1970; Zoutendyk, 1990; Dubber et al., 2004) and pleopodal setae are fresh for egg attachment (Booth, 2006). Females attach berry (eggs) onto these setae (Dubber et al., 2004). Development of the embryo happens in several visible stages (Silberbauer, 1971). After the brood period of approximately three months, larvae hatch in austral spring to summer (Silberbauer, 1971). The WCRL, like other palinurids, has a long and complex larval phase. In short: the short-lived (hours) naupliosoma larva hatches (Booth, 2006) followed by an extended planktonic larval phase (MacDiarmid, 1985). This so-called phyllosoma phase comprises of 11 stages of development and takes up to 7 - 8 months (Booth, 1997, 2006; Dubber et al., 2004; Kittaka, 1988). Thereafter, the non-feeding puerulus (post-larva) links the larval- with the juvenile phase (Figure 2). The puerulus actively swims inshore where moults into a juvenile after 10 days of pigmentation (Dubber et al., 2004; Booth, 2006). From there, juvenile lobsters moult and grow fast (Dubber et al., 2004; Hazell et al., 2001a). Growth of the WCRL is achieved via shedding of the old restrictive exoskeleton. It takes the female around 3 - 7 years before reaching sexual maturity while males from the same area reach sexual maturity at a similar or smaller size than females (Beyers and Goosen, 1987;

Booth, 2006). Juvenile males and females have similar growth rates until they reach maturity, after which the females' moult increment is smaller as more energy is diverted for gonad production rather than growth. An age-specific-, rather than size-specific relationship exists with regard to the onset of sexual maturity in the female (Beyers and Goosen, 1987). After reaching maturity, WCRL moult annually. Males moult in late spring to early summer (September-December), well before females in late austral autumn to early winter (April-June) and the mating period in winter (July-August)(Heydorn, 1969).

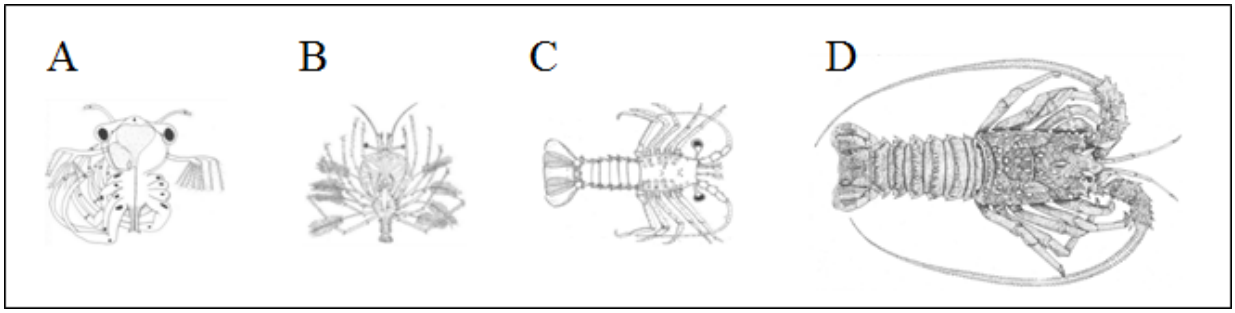

Figure 2. Depiction of some life stages of *J. lalandii*: A) nauplisoma, B) phyllosoma stage 11, C) puerulus and D) juvenile/adult. Pictures A-C from Silberbauer (1971) and D from Holthius (1991).

For the understanding of reserve accumulation for growth, it is necessary to understand and consider the moult cycle. The moult cycle of decapod crustaceans consists of four phases. These are post-moult (AB), inter-moult (C), pre-moult (D) and ecdysis (the actual moulting), based on microscopic differences in the integument (Drach, 1939). These integumental changes can be observed microscopically in clippings of pleopods (Figure 3). For stage C, a further split into substages C_1, C_2, C_3 *vs*. C_4 was suggested (Charmantier-Daures et al., 2004) and for *J. lalandii* existence of substage C_4 was hypothesised, based on hormone titres only (Marco, 2012). Stage D can be divided into D_0 - $D_{3\text{-}4}$ of which D_1 is further divided into D_1', D_1" etc. (Charmantier-Daures et al., 2004). These phases are underpinned by the titres of antagonistic hormones (ecdysteroids and moult-inhibiting hormone, MIH) in the haemolymph (Gäde and Marco, 2006). While phase AB lasts only a few days in adult WCRL, the remaining duration is almost equally divided between stages C and D with indications that stage D is somewhat longer than stage C (Marco, 2012). Substage C_4 is described as "consolidation" phase, in which the integument pauses from development, and ends with the detachment of the epidermis from the cuticle (marking the start of D_0). Reserves are accumulated in this substage, especially in the hepatopancreas (Charmantier-Daures et al., 2004). In *J. lalandii* fisheries research, moult stage was previously, if at all, recorded on the basis of shell hardiness and stages were subdivided into Hard, Hard Old, Soft New, and Hard New (Heydorn, 1969). This method is relatively subjective and needs some experience. Another, more accurate method, is the

setagenic analysis of pleopods by microscope. It distinguishes the moult stages by analysis of the developing new epidermis, especially with regards to its setae (Drach, 1939). Yet another method is the analysis of haemolymph moult hormone titres as described for adult *J. lalandii* by Marco (2012).

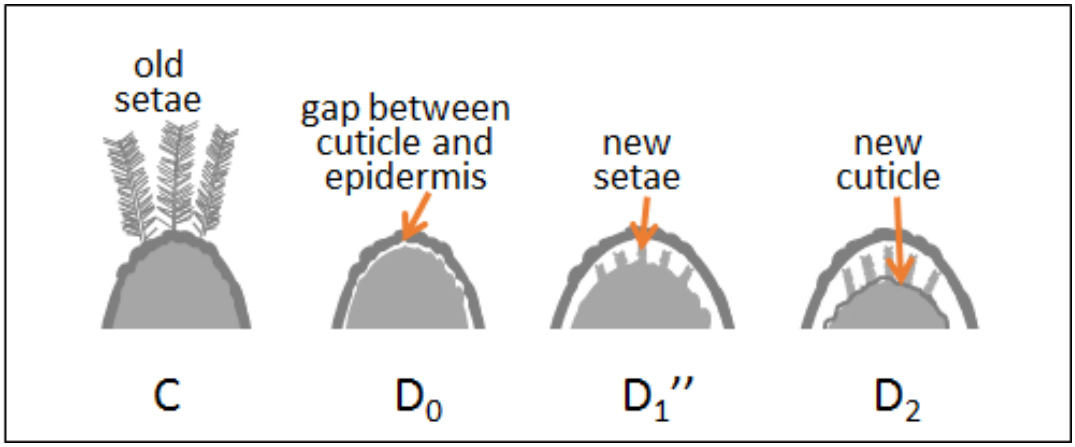

Figure 3. Schematic depiction of the most frequent moult stages of *J. lalandii* observed in the present project: A) stage C, B) stage D_0, C) stage D_1", D) stage D_2. During stage C, setal walls and cuticle thicken progressively; in stage D_0 a retraction of the epidermis creates a gap; in stage D_1" new setae appear; in stage D_2 new setae have grown and a new cuticle forms. According to Isaacs et al. (2000) and Marco (2012).

The process of ovarian maturation in female WCRL is closely synchronised with the moult cycle as well as with the respective cycles of the opposite gender (Figure 1). It is therefore essential to consider this development when estimating stock development. After reaching maturity, WCRL reproduce in an annual cycle. Male gonads (testes) are very small, and negligible as an energy depot. In females, however, final weight of the gonads (ovaries) can reach 7% of total body weight (own observation) as reserve metabolites – such as lipids and proteins – accumulate in the eggs (Harrison, 1990). Like in other decapod crustaceans, a new maturation cycle of the ovaries of *J. lalandii* commences after spawning, which is evident by growth in size and a colour change of the ovaries (Heydorn, 1969). In WCRL females, progress of ovarian maturation can be split macroscopically into stages ranging from 1 to 6 by ovarian size, weight and colour (Heydorn, 1969). There are also histological (Heydorn, 1969) and biochemical methods (Teshima et al., 1989; Mourente et al., 1994) of staging in decapod crustaceans. In the field, however, the macroscopic staging is the method of choice due to its simplicity and practicality. Some more detail on aspects of the biology of WCRL is provided in the following section where they are discussed in the context of reserve accumulation for growth and reproduction.

2.3 The role of lipids in reserve accumulation for growth and reproduction

Research on decapods has so far provided the bulk of knowledge on crustacean metabolism in general, including that of depot lipids (Sánchez-Paz et al., 2006). Although it is not clear as to what extent lipids are a nutritional requirement (Sánchez-Paz et al., 2006), lipids are certainly the main organic body reserve for most crustaceans (Phillips, 2006). In general, main energy metabolites are lipids and proteins but although proteins were previously regarded as the principal energy source in crustaceans (New, 1976), more recent research revealed that crustaceans differ widely in this regard and that lipids play a major role (Sánchez-Paz et al., 2006). Carbohydrates reserves (in the form of glycogen) play a minor role: they are limited in crustaceans and only power short-term high-intensity anaerobic muscle work, such as tail-flip, after phosphagens are used up (England and Baldwin, 1983). In addition, carbohydrates provide structural chitin building blocks (N-acetylglucosamine) for the formation of the exoskeleton, which are not available for energy production (Charmantier-Daures and Vernet, 2004). The preferred compound and the sequence of deposition and depletion thereof vary considerably between decapod crustacean species (see Sánchez-Paz et al., 2006). Lipids, as the most chemically reduced (i.e. less oxidised) organic molecules, can be stored in substantial amounts. They are catabolised to provide energy for various processes such as growth, moulting and reproduction (Sánchez-Paz et al., 2006). Also, in contrast to penaeid shrimps, proteins and lipids cannot easily be substituted by carbohydrates in palinurid feed: Spiny lobsters digest some carbohydrates well but utilise them inadequately (Rodriguez-Viera et al., 2017). Due to their importance for palinurid growth and reproduction, the role of lipids in reserve accumulation and use are discussed in further detail here.

Our understanding of the lipid metabolism of palinurids is poor in comparison to that for other decapod species used in aquaculture. Due to the paucity of relevant palinurid data, information is often drawn from other decapod infraorders, such as the closely related Astacidae (clawed lobsters) and the Penaeidae (shrimps, prawns). However, interpolation of such data is problematic. Palinurids belong, together with Scyllaridae (slipper lobsters), to the infraorder Achelata (Wolfe et al., 2019). They have a unique and completely different life cycle (notably their complex and protracted larval phase), demanding different metabolic requirements than related decapod taxa (Perera and Simon, 2015).

The high levels of depot lipids in palinurids, especially in the hepatopancreas (the main organ for lipid digestion and accumulation) indicate their importance in many biological processes (Gibson and Barker, 1979). A more detailed examination of the metabolism of depot lipids in

palinurids, considering their specific biology, is therefore desirable. The paragraphs below aim to summarize currently available data on the role of depot lipids in the biology of palinurid decapods. Although the focus is on adults, reference is made to juvenile palinurids and other decapod infraorders where there are no adult palinurid data were available.

Lipid dynamics during biological cycles

The biological cycles of post-larval palinurids are complex with substantial variation between species both pre and post attainment of sexual maturity (for reviews, see Lipicus and Eggleston, 2000, George, 2005). Moult- and reproductive cycles of males and females are mostly seasonal and tightly coupled in some species (see Figure 1 for *J. lalandii*). On the physiological level this is reflected in depot lipid dynamics of both genders. Adult males moult well before females and are in intermoult (hard) stage (C) at mating (Lipcius, 1985). Mating takes places a certain period after female ecdysis, but the period differs from a few days to weeks between species (Lipicus and Eggleston, 2000).

The Southern African silent *J. lalandii*, for example, adopts an annual moult cycle after attainment of sexual maturity (Newman and Pollock, 1974), after which male and female moult- and reproductive cycles are tightly synchronised (Figure 1). Adult male *J. lalandii* moult in late austral spring to early summer (September-December), well before females in late autumn to early winter (April-June) and the mating period in winter (July-August). At the time of oviposition and spawning, the female exoskeleton is still relatively soft (Heydorn, 1965, 1969; Berry and Heydorn, 1970; Zoutendyk, 1990; Dubber et al., 2004) and pleopodal setae are fresh for egg attachment (Booth, 2006). As a result of this synchronisation, lipid- and other metabolic reserves in the male hepatopancreas and female gonads build up during the same period in *J. lalandii* (Cockcroft, 1997; Heydorn, 1969) and a relationship of male growth and female fecundity was shown (Melville-Smith et al., 1995). Environmental influences therefore impact both simultaneously (Cockcroft, 1997), most likely via affecting the build-up of lipid depots.

The substantial energetic cost of reproduction is the major factor difference in the energy budgets of adult males and females. Until they reach maturity, juvenile males and females have similar growth rates. Thereafter, females' moult increment is reduced to provide more energy for gonad production. In large reproductive *J. lalandii* females, only 5% of somatic production is used for growth and 95% are allocated to reproduction (Zoutendyk, 1990). Gonad size of males is minute and therefore most of somatic production is apportioned to growth (99% in *J. lalandii*, Zoutendyk, 1990). In times of nutritional stress *J. lalandii* females sacrifice growth to

optimise egg production (Cockcroft, 1997). As detailed below, the main metabolite to fuel growth and reproduction in palinurids are lipids.

Lipids stored in palinurid decapods

Like other decapods, palinurids contain high concentrations of lipids which are mainly concentrated in the hepatopancreas, ovaries and abdominal muscle (Table 1). Lipid concentration in some of these tissues varies with moult- and reproductive cycles. The dominant lipid classes (see Figure 4 for structures) are triacylglycerides (TAG), phospholipids (PL) and sterols (ST).

Table 1. Overview of depot lipids in the main storage organs of palinurid crustaceans.

	Total Lipid content*	Lipid composition	
	(% dry weight)	lipid classes (% of total lipids)	fatty acid profile (dominant FAs)
Hepatopancreas	max. 70-75%	TAG: 90% ST: < 1% PL: < 1.7%	saturated and unsaturated long-chain FA
Abdominal muscle	0.2-2%	TAG: 1% ST: < 5-8% PL: ~ 90%	saturated and unsaturated long-chain FA
Ovaries	~40%	TAG: 45% ST: < 3-4% PL: 50%	saturated and unsaturated long-chain FA

Triacylglycerols: There is very little information on TAG contents and dynamics in mature palinurids but data from post-larval animals (i.e. pueruli and juveniles) reveal that the overall concentration increases during ontogenesis until TAGs (Figure 4A) become the dominant lipid class (Jeffs et al., 2002). In the pelagic stages, TAG content is very low and instead, PLs in the fat body (located laterally in the abdomen) serve as depot lipid for energy provision. This seems to be an adaptation to a pelagic/nectonic life where translucent PLs ensure camouflage in contrast to the opaque TAGs (Jeffs et al., 2001). After settlement, PLs from the fat body are rapidly converted to TAG and deposited in the developing hepatopancreas for the moult to juvenile stage (Jeffs et al., 2001).

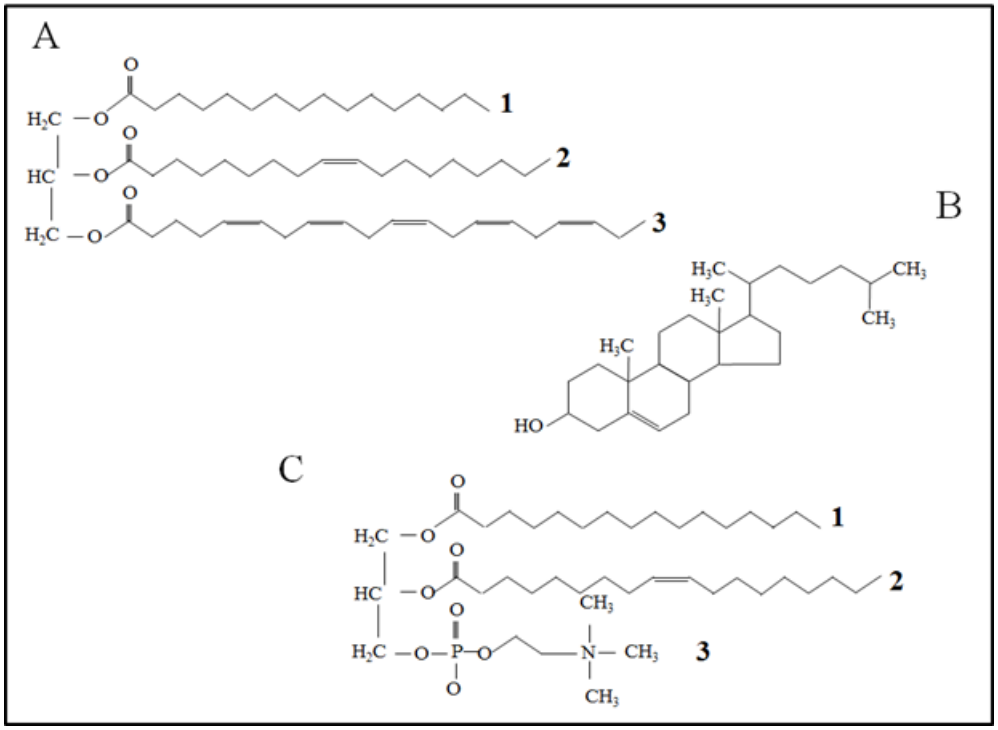

Figure 4. Primary chemical structures of main depot lipids in palinurids: A) a Triacyl-glyceride with dominant fatty acids found in palinurids as residues 1) palmitic acid ($C_{16:0}$), 2) oleic acid ($C_{18:1}$ and 3) EPA ($C_{20:5(n-3)}$); B) Cholesterol (the dominant sterol) and C) Phosphatidyl Choline, the dominant Phospholipid, with the saturated fatty acid 1) palmitic acid and the unsaturated fatty acid 2) oleic acid as residues and 3) the phosphate group with Choline residue. Note: Secondary and tertiary structures not taken into consideration.

Sterols: Cholesterol (Figure 4B) constitutes 90-95% of total sterols in crustaceans (Kanazawa, 2001) and is organ-specific in palinurids (Smith et al., 2004). It is also the dominating sterol in *J. lalandii* hepatopancreas with only traces of other sterols present (Ligthelm et al., 1953). Cholesterol plays an important role as a cell constituent and as a metabolic precursor of steroid hormones and moulting hormones (Harrison, 1990). Spiny lobsters are, like crustaceans in general, unable to synthesise sterols *de novo* but are able to convert some sterols to cholesterol (Teshima and Kanazawa, 1971) and therefore need to obtain sterols from their diet: A lack of dietary cholesterol was shown to lead to mortality in clawed lobsters when dietary levels were too low (Kean et al., 1985). Food levels of levels of 0.2-2.0% cholesterol are regarded as optimal for a range of crustacean species (Kanazawa, 2000).

Phospholipids: In general, the polar PLs (Figure 4C) are components of membrane structures and mediators and modulators of transmembrane signalling. In crustaceans, certain phospholipids are necessary as constituents of lipoproteins (High density lipoproteins (HDL) and very high-density lipoproteins (VHDL)) which play an important transport role during mobilisation of lipids to and from the hepatopancreas (Kanazawa et al., 1985; Lee and Puppione, 1978; Yepiz-Plascencia et al., 2000; Yepiz-Plascencia et al., 2002).

Phosphatidylcholine is a particularly important PL because it is an essential component of these lipoproteins (Hertrampf, 1992). Phospholipids play a critical role in lobster growth and development which is attributed to the lobsters' (crustaceans in general) limited ability to biosynthesize phospholipids from fatty acids (Kanazawa et al., 1985). Lobsters therefore need to obtain PLs from their diet. Phosphatyl Choline (PC) is the dominant PL in *J. lalandii* hepatopancreas with a ~60% content and ~71% in ovaries (de Koning and McMullan, 1966).

Fatty Acid composition of storage lipids: The fatty acids (FA) α-linolenic acid (ALA, $C_{18:3(n-3)}$, linoleic acid (LA, $C_{18:2(n-6)}$, Eicosapentaenoic acid (EPA, $C_{20:5(n-3)}$, Docosahexaenoic acid (DHA, $C_{22:6(n-3)}$ and Arachidonic acid (AA, $C_{20:4(n-6)}$ are essential (EFA) for growth and survival of crustaceans in general and cannot be synthesised *de novo* (Kanazawa et al., 1979; D'Abramo, 1997; Querijero et al., 1997; Glencross and Smith, 2001). There is little information around the role and function of these specific EFA in spiny lobsters (Williams, 2007; Glencross, 2009), however, during starvation of post-puerulus *Panulirus cygnus* (DHA, EPA, AA; Limborn et al., 2008) and ontogenesis of *Jasus verreauxi* (post-larvae, LA, DHA, EPA; Jeffs et al., 2002), these EFA are spared from catabolism at the cost of non-essential FA. The same seems to be true in the hepatopancreas and the leg muscle during increased metabolic activity of *J. edwardsii* due to environmental change (EPA, Chandrapavan et al., 2009). In *J. lalandii* hepatopancreas, the main fatty acids are saturated C_{14}, C_{16}, C_{18}, C_{20}, C_{22} and unsaturated C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26} (Ligthelm et al., 1953). Long chain fatty acids also dominate the fatty acid profile of ovary lipids (Smith et al., 2004).

Dietary Sources of depot Lipids

Palinurids selectively prey on a diverse assemblage of benthic and infaunal species such as molluscs (gastropods, bivalves), other crustaceans (including cabs, barnacles, mysids), echinoderms, polychaetes, corraline algae and sponges (Barkai and Branch, 1988; Lipcius and Eggleston, 2000). Cannibalism is also relatively common palinurid lobsters (Booth and Kittaka, 1994; Barkai and Branch, 1988). Dominant components of the diet of most palinurid species seem to be mytilid mussels (genera *Mytilus, Aulacomya, Perna*), sea urchins and other crustaceans. The ribbed mussel *Aulacomya ater*, for example, can constitute up to 97% of the stomach content of *J. lalandii* (Pollock, 1979). In many experiments, feeding mussels achieved maximum growth and lipid accumulation in the hepatopancreas when compared with all other natural and artificial diets (Glencross et al., 2001; Esterhuizen, 2004; Ward and Carter, 2009; Simon and Jeffs, 2013). The abundance and distribution of mussels has been directly linked to

lobster growth rates in the field (Field and Wickens, 1988; Beyers and Goosen, 1987). A reason for the importance of mytilid mussels as dietary source for palinurids is, apart from their high total lipid- and phospholipid content, their high content of some long-chained polyunsaturated fatty acids such as EPA, DHA and ARA (Kariotoglou and Mastronicolis, 1998). These dietary fatty acids are essential for development and growth of the lobsters during critical stages of their growth cycle (see above, Esterhuisen, 2004). Sea urchins are also a rich source of nutritional lipids: Urchin gonads are not only regarded as a reproductive organ but also a metabolite storage site that contributes 10-15% of total biomass and contains about 5% total lipids, which are rich in essential FA such as DHA, ARA and EPA (Liyana-Pathirana et al., 2002; Arafa et al., 2012). Crustacean prey species also provide a rich source of dietary lipids.

Lipid storage sites

Major storage sites of depot lipids in decapods, including palinurids, are the hepatopancreas, female gonads and muscle tissue (Figure 5). The amount and types of lipid stored change during the moult and reproductive cycles (see, for example, Harrison 1990; Sánchez-Paz et al., 2006). These changes are interlinked, are impacted by environmental influences (including food availability) and are under endocrine control.

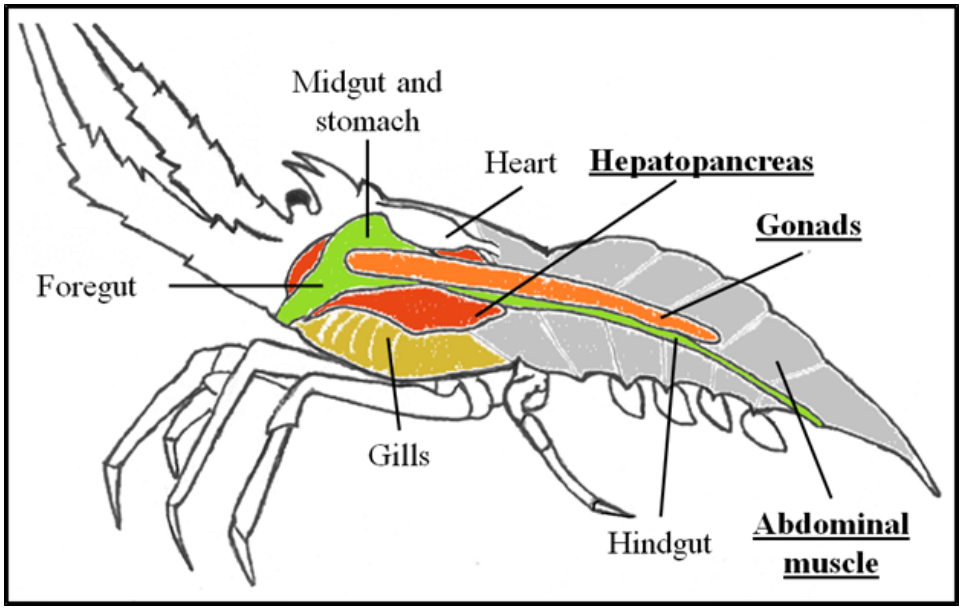

Figure 5. Schematic location of relevant organs for lipid storage (**bold underlined**) in spiny lobsters.

The Hepatopancreas (or digestive gland), is a central organ in the biology of decapods and serves vital metabolic functions such as enzyme synthesis and secretion, uptake and storage of nutrients and their precursors, synthesis and catabolism of storage metabolites, excretion, provision of material and energy for moult and growth, etc. (Gibson and Barker, 1979). It is typically high in lipids and is the principal organ for their digestion, absorption, storage,

synthesis and distribution (Gibson and Barker, 1979). The palinurid hepatopancreas has a particularly high lipid content which can reach 70-75% of dry weight in the late pre-moult stage (Cockcroft, 1997; McLeod et al., 2004; Smith et al., 2004). Relative hepatopancreas size and its lipid levels respond rapidly to changes in physiological and environmental parameters. Lipid depots are used up quickly in response to environmental and physiological changes such as moult (Cockcroft, 1997) and dietary stress (Smith et al., 2004).

The palinurid hepatopancreas starts to form after settlement, when PL from the so-called fat body of the puerulus (post-larva) are converted into TAG and moved to the hepatopancreas for storage and mobilisation during the subsequent moult (Nishida et al., 1995; Pearce, 1997; Jeffs et al., 2001). The fat body seems to become redundant thereafter. In adult spiny lobsters, the main lipid class in the hepatopancreas are the TAGs (Ligthelm et al., 1953; Smith et al., 2004) where their contribution can be as high as about 90% of total lipids (TL)(Smith et al., 2004). TAGs of the hepatopancreas seem to be the preferred lipid class for energy provision: Their share declined concomitantly with a decline in TL during starvation (Smith et al., 2004). In contrast to other organs, ST concertation is less than 1% in palinurid hepatopancreas (Smith *et al.* 2004). The principal ST in *J. lalandii* is cholesterol (Ligthelm et al., 1953). PL are also very low (0.4 – 1.7% PL (Ligthelm et al., 1953; de Koning and McMullan, 1966; Smith et al., 2004). Phosphatyl Choline (PC) is the dominant PL in *J. lalandii* hepatopancreas (~60%)(de Koning and McMullan, 1966) and plays an important role in the survival and growth of the lobster. This is attributed to selective fatty acids such as linoleic-, linolenic-, EPA- and DHA fatty acid groups (Kanazawa and Koshio, 1994).

In decapod hepatopancreas, lipids are at their minimum immediately after ecdysis and are starting to accumulate again at the end of the postmoult (stages A-B) through intermoult up to stage C_4 and onset of premoult (D_1). Later in premoult stage (from D_2) lipid levels decrease rapidly, coinciding with non-feeding in stages D_2'–B around ecdysis (Charmantier-Daures and Vernet, 2004). Lipids therefore built up in the hepatopancreas in preparation of the moult until the late intermoult (lipid peak) phase after which reserves are used/redistributed for processes related to ecdysis. In male *J. lalandii*, the peak lipid storage period has a positive correlation to the measured growth (moult) increment and can be used as a reliable potential indicator for lobster growth rate (Cockcroft, 1997). A lack of lipid accumulation during intermoult by this species was shown to lead to low growth rates and even negative growth (shrinkage)(Cockcroft and Goosen, 1995; Cockcroft, 1997). The relative size of the hepatopancreas (Hepatosomatic Index = HSI) changes mainly with the dynamics of depot lipids: Both peak around July to August in male *J. lalandii*, i.e. short before male moult (Cockcroft, 1997).

Studies on the lipids in organs of female palinurids have received less attention. The timing and amount of the lipid present in the female hepatopancreas differs from that of males. Lipid levels remain low during ovarian maturation and peak shortly after HSI reaches its maximum. They remain low during the berry cycle and recover only slightly thereafter. In *J. lalandii*, this is in July and October to January, respectively (van Rooy, 1998).

Female Gonads (Ovaries): Whereas male gonads are very small and negligible as energy depot, the ovary becomes an additional centre for lipid metabolism during ovarian maturation in decapod crustaceans including palinurids. Lipids accumulate together with proteins and the GSI (Gonadosomatic Index) increases (Harrison, 1990). Lipids in gonads accumulate until spawning and the GSI increases concomitantly (van Rooy, 1998). As a result, ovaries contain high concentrations of total lipids (TL) reaching approximately 12.5% (wet weight) in *J. lalandii* (de Koning and McMullan, 1966) and 41% (dry weight) in *J. edwardsii* (Smith et al., 2004). TAG contribute approximately 45% of TL whereas ST content (~3-4% of TL) is higher than in hepatopancreas but lower than in abdominal muscle (Smith et al., 2004). The increased storage of ST (cholesterol) during maturation most likely serves incorporation into egg- and embryo membranes (Harrison, 1990). Palinurid ovaries are particularly high in PL (about 50% of TL, Ligthelm et al., 1953; de Koning and McMullan, 1966; Smith et al., 2004) which comprise mostly of PC (~71%) and PE (23%) whilst their fatty acid profile consist mostly of long chain fatty acids such as palmitic acid ($C_{16:0}$) DHA ($C_{22:6}$) and EPA ($C_{20:5}$)(de Koning and McMullan, 1966).

Dynamics of lipids in decapod ovaries and hepatopancreas are interlinked. The lipid accumulation in decapod ovaries is a result of vitellogenesis (production of vitellogenin and lipoprotein and storage by oocytes) in the ovaries themselves but possibly also with contribution from the hepatopancreas from where it is subsequently transferred to the ovaries via the haemolymph (Harrison, 1990). In palinurids, however, there is indirect evidence for *J. edwardsii* that lipid demand of ovarian maturation is to a large extent met through dietary intake and not only (or not at all) from the hepatopancreas (Smith et al., 2004) as it was previously found in several crab species (Hasek and Felder, 2005, 2006). Changes in the composition of lipids concerning lipid classes and FA during maturation, such as documented for crabs (Mourente et al., 1994), are yet unknown for palinurids. Ovarian maturation and reserve built-up depend on food supply: Starvation leads to reduction of gonad index (GSI) and lipid content in *J. edwardsii* (Smith et al., 2004).

Abdominal Muscle: Although the abdominal (or tail) muscle is considered an energy reserve in most crustaceans, it is not utilised as a major energy source by male *Jasus lalandii* during a

routine (non-food limited) moult cycle. The lipid content is low between 0.2 - 2% and changes little during the growth periods (Cockcroft, 1997). It was therefore concluded that abdominal muscle reflects more long-term changes and responds less sensitively to environmental and physiological changes experienced in the field (Cockcroft, 1997). In adult male *J. edwardsii*, however, lipid content almost halved while proteins remained constant after a 28-day starvation period (McLeod et al., 2004). This indicates a lipid storage function of the abdominal muscle and the consumption of lipids to preserve (structural) proteins during periods of extended nutritional stress. The tail muscle constitutes a high portion of the total mass off a palinurid, for example 45% (with exoskeleton) in *P. gilchristi* (Groeneveld and Goosen, 1996). This compares to a maximum 5% of the hepatopancreas at peak accumulation in of most palinurids. Therefore, the total amount of lipids in the tail should not be neglected. In contrast to the hepatopancreas, where TAG form the bulk of TL, the tail is dominated by PL with levels of about 90% (Smith et al., 2004; Nelson et al., 2005; Chong Shu-Chien et al., 2017) and TAG content is low at about 1% (Smith et al., 2004). In addition, sterol concentration is highest in abdominal muscle (5-8% in *J. edwardsii*; Smith et al., 2004). It can therefore possibly be regarded as a cholesterol storage site like it was suggested for prawns (Kanazawa et al., 1988).

Lipid Metabolism

Before any dietary lipids can be metabolised, food items of spiny lobsters are mechanically destructed by their mouthparts before they are ingested into the foregut. The latter contains a gastric mill that further crushes and mixes food particles (Johnson and Hooper, 1992). The volume of the foregut is only 2-3% of body weight and hence, food consumption is limited to an estimated 1 foregut per day (Perera and Simon, 2015). Quality food is therefore essential for optimum growth and reproduction and may explain the high ratio of mytilids in the diet of spiny lobsters as they have high lipid content and are rich in essential FA and phospholipids. Lipid digestion (TAG and PL) is facilitated by gastric lipases produced and secreted by the F-cells of the hepatopancreas, although these lipases have not been characterised so far in terms of triglyceride hydrolase- and phospholipase activities (Perera and Simon, 2015). High content of PLs is positive due to their high solubility and digestibility and PLs may also enhance transport and retention of TAG and ST (Coutteau et al., 1997). In the gastric space, neutral lipids are cleaved into mono- and diglycerides and then converted to phospholipids by the B-cells of the hepatopancreas and absorbed together with FA (Chang and O'Connor, 1983; Ceccaldi, 2006). From here, they are released and transported in the haemolymph for use in organs or for conversion to TAG for storage (Chang and O'Connor, 1983). Phospholipids are

the main circulating lipids in crustaceans (Chang and O'Connor, 1983). This is due to their role as part of lipoproteins: HDL and VHDL play an important role in the transport of lipids during mobilisation of lipids to and from the hepatopancreas (Kanazawa et al., 1985; Lee and Puppione, 1978; Yepiz-Plascencia et al., 2000, 2002). Phosphatidyl Choline is a particularly important PL because it is an essential component of these lipoproteins (Hertrampf, 1992). For energy production, first-step enzymes (lipases, esterases) mobilise stored TAG and PL. These enzymes are usually under endocrine control; however, exact details on such regulation in decapods are unknown as yet. Fatty acids liberated from these TAGs and PLs are catabolised via beta oxidation in the target organs, such as muscle. Activity of the beta oxidation enzyme HOAD (3-hydroxyacyl-CoA dehydrogenase) is positively correlated with the increasing role of lipid stores during ontogenesis in *J. edwardsii* (Wells et al., 2001).

Endocrine Control of depot lipids

The dynamics of depot lipids in palinurids are driven by food availability and are under endocrine control. In general, moulting (i.e., growth) and reproduction in decapod crustaceans are under complex endocrine control by respective antagonistic hormones. Briefly (and not comprehensively): Ovarian maturation, including vitellogenesis, is stimulated by the release of the gonad-stimulating hormone (GST) from brain and thoracic ganglion, synthesis of which is inhibited, in turn, by the vitellogenesis-inhibiting hormone (VIH) produced in and released from the sinus glands of the eye stalks (Nagaraju, 2011). So far, no unique structures of a VIH (of the cHH/MIH/VIH group of peptides) have been found in palinurids (Marco and Gäde, 2006) but VIH activity was shown for known cHHs of *J. lalandii* (Marco et al., 2002).

Moulting, which is necessary to adjust the exoskeleton to a larger body and allow for growth, is initiated by the steroid moult hormone (ecdysteroid) which is produced in the Y-organ and whose antagonist is the moult-inhibiting hormone (MIH) produced in the sinus glands (Carlisle and Knowles, 1959). A palinurid ecdysone (20 α-hydroxy ecdysone) was discovered in *J. lalandei* (now *J. edwardsii*; Passano, 1960; Hampshire and Horn, 1966) and *J. lalandii* (Marco et al., 2001), whereas structures of MIH in palinurids are known for *J. lalandii* and *J. edwardsii*, respectively (Marco et al., 2000a, 2001; Christie and Yu, 2019). Endocrine control of growth and reproduction also determine the lipid aspect of these two processes. Suppression of moult by MIH allows for the accumulation of lipids in the hepatopancreas, whereas GST allows for the synthesis and accumulation of lipids in the ovaries during the respective phases in the moult- and ovarian cycles. There is no published information to date on the direct endocrine

control of lipid synthesis or catabolism by these hormones in palinurid crustaceans, or in fact in decapods in general.

Food consumption in palinurids is of course regulated by the availability of food items of the sufficient quality and quantity (Perera and Simon, 2015). Food intake in decapods is also under hormonal control by the neuropeptide NPF which was shown to increase food intake, leading to faster growth, in penaeid shrimps (for review see Christie, 2011). Two members of the NPF family were recently found in *Sagmariasus verreauxi* and *J. edwardsii* but an effect on food uptake has yet to be demonstrated (Ventura et al., 2014; Christie and Yu, 2019). In addition, eyestalk ablation, elimination of MIH and CHH (see above), increase food intake and growth in the spiny lobsters *Panulirus homarus* (Radhakrishnan and Vijayakumaran, 1984; Vijayakumaran and Radhakrishnan, 1984) and *P. ornatus* (Juinio-Meñez and Ruinata, 1996). This suggests a role of eyestalk neuropeptides in regulation of food intake, since NPF seem to be produced by various nervous organs (Christie, 2011).

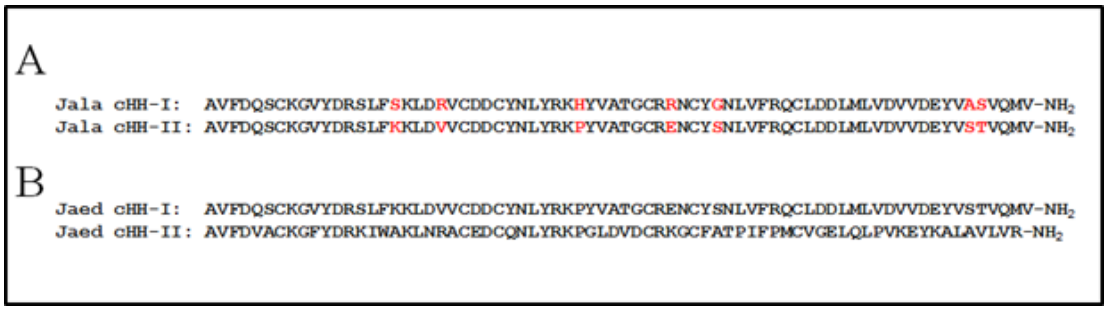

Figure 6. Primary structures of cHH hormones, potentially involved in lipid metabolism in the palinurids A) *J. lalandii* and B) putative hormomes in *J. edwardsii* derived from BLAST analysis of genome.

Members of the so-called hyperglycaemic hormone family (cHH) were shown to mobilise carbohydrates in palinurids (Marco and Gäde, 1998; Marco et al., 2000b; see Figure 6 for structure); however, hyperlipaemic activity was only shown so far in three infraorders of decapods: crabs, freshwater crayfish (Santos et al., 1997) and shrimps (Camacho-Jiménez et al., 2017; Montiel-Arzate et al., 2020). Haemolymph levels of Total Lipids, TAG, PL and FA were positively affected. In addition, in a freshwater crayfish, cHH caused the release of PL and FA from hepatopancreas *in vitro* (Santos et al., 1997). A similar hyperlipaemic activity of the known cHH peptides is likely in palinurids but has not yet been reported.

2.4 Exploitation and management of the WCRL resource

Exploitation of the WCRL resource started in the late 1900s and peaked in the early 1950s at about 18000 t (Cockcroft and Payne, 1999; Johnston and Butterworth, 2005). Catches levelled off at around 10000 t between the late 1950s and the 1960s (Cockcroft and Payne, 1999) and in the 1980s, the resource supported a commercial industry with relatively stable annual catches of 3500 – 4000 t (Johnston and Butterworth, 2005). This stability ended in the early 1990s when somatic growth rates decreased sharply, causing a negative impact on the recruitment of lobsters (Cockcroft, 1997). In 1999, the harvestable component of the resource was estimated to be at 5% and spawning biomass at 20% of pristine levels (i.e. pre-exploited levels, 1910)(Cockcroft and Payne, 1999). Currently, the harvestable component is estimated to be at 1.8% of pristine levels (DEFF, 2020).

Currently, an annual Total Allowable Catch (TAC) for male WCRL (exclusively) is set scientifically, guided by an Operational Management Procedure (OMP, see below). According to this, the resource is managed by several commercial and non-commercial sectors (offshore-, nearshore-, subsistence fishermen, small scale fishers) and recreational sectors (DEFF, 2020). Management controls also include protection of females with eggs (berried females) and soft-shelled lobsters (freshly moulted), a closed season, closed areas and a daily bag limit for recreational fishers (DEFF, 2020).

To achieve sustainability and rebuild the stock by 2021 by 35% above the 2006 level, an operational management procedure (OMP) for scientific TAC recommendations is in use since 1997 (DEFF, 2020). According to this OMP, the Total Allowable Catch (TAC) for a new season is currently determined exclusively from Catch per Unit Effort (CPUE), a fisheries independent abundance index resulting from annual monitoring surveys (FIMS), growth data of the previous season as well as estimates of catches of recreational-, small-scale- and related fisheries (DEFF, 2020). Most recently, an additional control of effort was included in the management of the resource (DEFF, 2020). The annual TAC is subdivided into fishing zones and areas (Figure 7). The two sampling sites for the present project reside in the same zone (Figure 7).

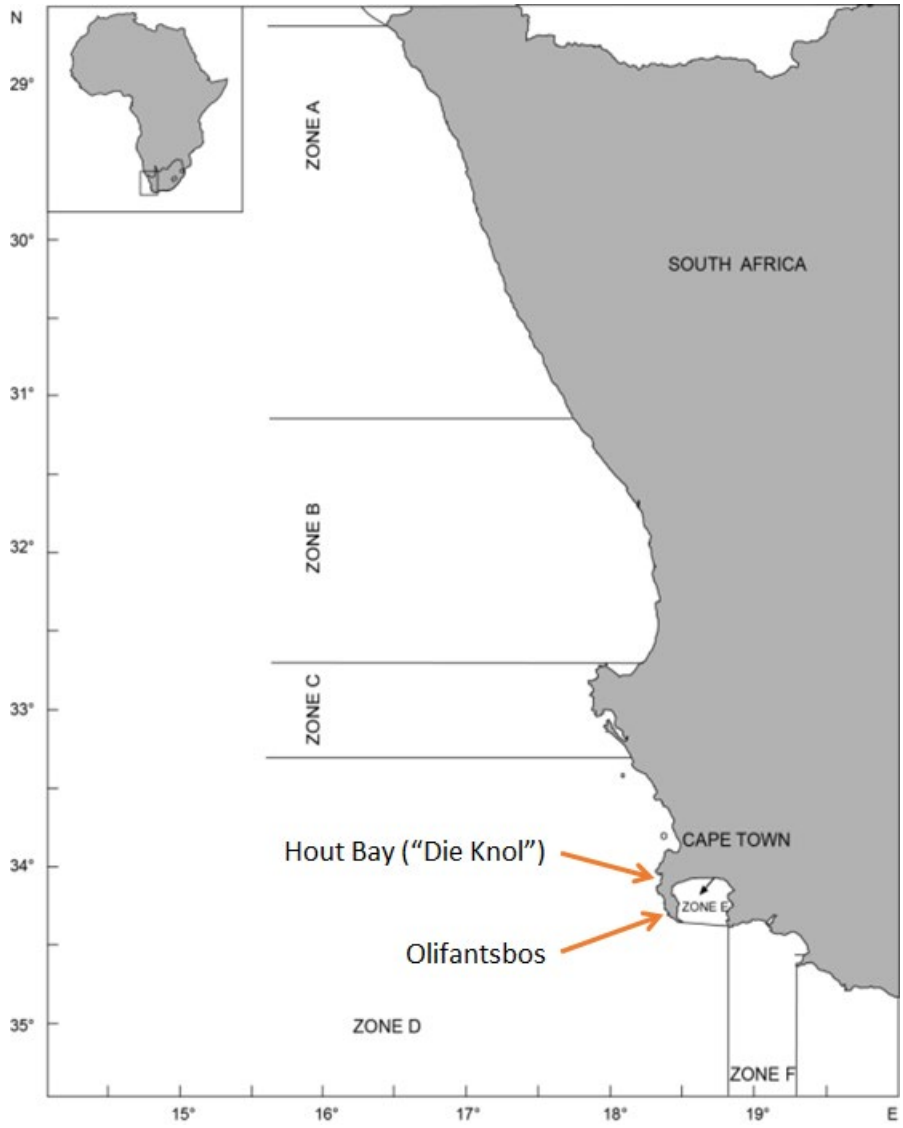

Figure 7. West Coast rock lobster fishing zones (reproduced with permission from A.C. Cockcroft (DFFE). The study area with the two sampling sites "Knol" in Hout Bay and Olifantsbos reside in Zone D (orange arrows).

The above-mentioned stock estimate is purely based on animal numbers and growth in the past but neglects the current physiological condition of the lobsters. This is needed, for example, in situations when lobsters shrank during their annual moult (when they should have grown) as an assumed result of unfavourable environmental conditions - especially food availability (Cockcroft and Goosen, 1995). Although effects of such events are taken indirectly into account via OMP procedures, they highlight the need to find additional, timelier, indicators to set the TAC for the next season. This would be novel in South African fisheries and even worldwide, attempts to use physiological indicators for setting catch amounts are very scarce.

2.5 Indicators for growth and reproduction

The OMP takes the impact of environmental effects on stock development indirectly into account. However, there is no component that captures physiological condition of lobsters. The ability to predict male somatic growth rate using a reliable physiological index would be a valuable improvement to the TAC setting process. Due to synchronization of male and female life cycles, metabolic reserves in the male hepatopancreas and female gonads build up during the same period in *J. lalandii* (Heydorn, 1969; Cockcroft, 1997) and a relationship of male growth and female fecundity was shown (Melville-Smith et al., 1995). Environmental influences therefore impact both processes simultaneously (Cockcroft, 1997). As a result, suitable indicators or predictors of male growth and, hence, stock development should exist in both sexes. This would be novel not only in South African fisheries but also worldwide where the use of physiological indicators as input data for setting catch levels is scarce.

For suitability as potential growth predictor, distinct variation of a parameter over the moult cycle or season is a key requirement for growth and reproduction (Cockcroft, 1997). Such indicators can be organ- or body part indices (in relation to each other or to total weight) and biochemical composition of organs. However, such indices are very species- and life stage-specific.

Body indices have been investigated to predict moult increment, for example hepatopancreas- and abdominal muscle size and composition as well as weight to carapace length ratio in male *J. lalandii* (Cockcroft, 1997). The carapace length serves as a stable reference value that does not change for the duration of a moult period. Weight to carapace length ratio (also termed Nutritional Index NTI) in juveniles of *Jasus edwardsii* (Oliver and MacDiarmid, 2001) and of *Panulirus argus* (Robertson et al., 2000), for instance, showed differences in response to feeding status and time under experimental conditions. The refractive index of haemolymph has also been used in palinurids, such as in juvenile *Jasus edwardsii* (Oliver and MacDiarmid, 2001), to determine nutritional status. Its disadvantage, however, is its strong correlation with protein concentration which, in turn, fluctuates strongly within a moult cycle, especially in premoult (Dall, 1974; Oliver and MacDiarmid, 2001). Attempts have also been made to use the ratios of lipid classes (triglycerides vs. cholesterol) in larval American (clawed) lobsters (Harding and Fraser, 1999). A lack of lipid accumulation during intermoult by the species was shown to lead to low growth rates and even shrinkage (Cockcroft and Goosen, 1995; Cockcroft, 1997). As in other decapods, the hepatopancreas is particularly relevant for many biological processes in palinurids and it is their main organ for lipid digestion and accumulation (Smith

et al., 2004; Perera and Simon, 2015). The depots in the hepatopancreas serve growth and the formation of the new exoskeleton. Therefore, the peak lipid storage period has a positive correlation to the measured growth increment and was proposed as a potential reliable indicator for growth in males, whereas for proteins, no such relationship was found (Cockcroft, 1997). Additional information on these processes in *J. lalandii* throughout fishing- and moulting seasons would aid understanding what determines growth increment at the next moult. This is because the current state of knowledge on direct and indirect environmental factors, such as food availability, that impact growth and reproduction of the WCRL is, as described above, suboptimal for predicting population- and resource development and, in turn, sustainable management. In particular, the impact during the reserve accumulation phase is important, because starvation or sub-optimal food supply may impact biological events (moult, spawning) long before they happen. Since the initial research on this topic (Cockcroft, 1997), no new information or confirmation of those results was provided.

2.6 Conclusion

The habitat of the WCRL is particularly variable in the short term and is impacted by climate change in the medium to long term. In addition, this invaluable fishing resource is under heavy exploitation pressure and the stock is critically low at 1.8% of pristine levels. It is therefore paramount to steady and rebuild the resource and manage it sustainably. Management tools, detailed in the OMP, exist but input data are currently suboptimal for predicting somatic growth, i.e., population- and resource development. In particular, knowledge on reserve accumulation for growth and reproduction in males and females are scarce but important. Such physiological and biochemical information can provide predictors long before natural and non-natural events impact growth and reproduction and, hence, stock development. Dynamics of organ lipid levels and, to a lesser extent, proteins are central to such knowledge in decapod crustaceans and palinurids in particular. Currently, there is no coherent body of information on all aspects of depot lipid metabolism in mature palinurids. Metabolism of depot lipids is a central aspect of palinurid biology and this review highlights the relative dearth of studies and information on this important topic. The results obtained will offer potential inputs for the OMP and, subsequently, ensure better management of the resource in times of a changing environment.

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Chapter 3

Analysis of biological and biochemical parameters of mature male spiny lobsters *Jasus lalandii* for identification of possible growth predictors

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Refer to Addendum B

Abstract

Biological body parameters of adult male West Coast rock lobsters, *Jasus lalandii*, of commercial size were analysed over two years to determine reserve accumulation during two moult (i.e. growth-) cycles. Two fishing areas were compared that were historically known for fast- and slow growth, respectively. In addition, setagenic development for moult stage determination and the biochemistry of the hepatopancreas were investigated from the fast growth area. This information was intended to confirm previously proposed predictors of annual male moult increment and identify potential new ones. Annual moult increments in these areas were determined from tag-and-recapture studies, including the seasons for which reserve accumulation was analysed. Data from the present study were not robust enough to confirm previously proposed predictors from peak accumulation months (March – May). Data arranged per moult stage were also unsuitable for prediction. Further results analysis, however, identified a subset of data that are potentially more useful: Hepatosomatic index HSI (+ 6%), lipid content (+ 9% w_W, + 12% w_D) and lipid per whole hepatopancreas (+ 24%) are all significantly higher in July 2011 than in July 2010, the last sampled months before the respective annual moult. These differences correlate with a 26% (0.7 mm) faster growth during subsequent moult in 2011 than in 2010. Aspects of hepatopancreas lipid metabolism in the months prior to moult have therefore the potential to provide simple and robust predictors of growth in adult male *J. lalandii*. However, more research is required that also includes the months adjacent to July in more than one area and for a longer period than two years.

Keywords: Spiny lobster, palinurid, physiological growth predictor, reserve accumulation, moult

3.1 Introduction

The West Coast rock lobster (WCRL), *Jasus lalandii,* is a slow-growing, cold- to temperate water palinurid species from the southern African Atlantic coast. Its fishery is one of the most important in South Africa due to its high value (2016 = R 538 m; ~$40 m) of which 98 % is exported (Fishing Industry Handbook, 2018). Additionally, the fishery is an important provider of employment for about 4 200 people, most of them along the South African west coast where impoverished communities live (DEFF, 2020). The Branch: Fisheries of the respective national governmental Department (currently Department of Environment, Forestry and Fisheries - DEFF) manages the West Coast rock lobster (WCRL) resource per zone (Figure 1) by means of Total Allowable Catch (TAC) and, more recently, by additional effort control. Other management measures include a defined fishing season and fishing areas, a minimum legal size and a ban on the retention of berried females (Cockcroft and Payne, 1999). Despite these measures, the resource remains under heavy fishing pressure (including substantial poaching) and is heavily depleted (DEFF, 2020). The current harvestable portion of the resource (males above 75 mm carapace length) is estimated to be only 1.8% of pristine levels (DEFF, 2020). Accordingly, annual catches have declined from a peak of 18 000 t in the early 1950s to about 1 000 t in 2019 (DEFF, 2020). In addition to fishing pressure, environmental influences have impacted the resource including reduced growth rates (Pollock et al., 1997), low-oxygen events that caused mass stranding of lobsters (Cockcroft, 2001) and an eastward shift in lobster distribution (Cockcroft et al., 2008).

To achieve sustainability and rebuild the stock, an operational management procedure (OMP) was introduced in 1997 (Cockcroft and Payne, 1999). The OMP uses abundance indices (commercial Catch per Unit Effort (CPUE) and a fisheries independent abundance index derived from annual monitoring surveys (FIMS)) and male somatic growth rate to set the TAC prior to the start of the fishing season (DEFF, 2020). While the physiological condition of lobster is taken into account indirectly by the OMP, the ability to predict male somatic growth rate using a reliable physiological index would be a valuable improvement to the TAC setting process. This would be novel not only in South African fisheries but also worldwide where the use of physiological indicators as input data for setting catch levels is scarce.

Such indicators can be organ- or body part indices (in relation to each other or to total weight) and biochemical composition of organs. However, such indices are very species- and life stage-specific. Attempts have been made, for instance, to use the ratios of lipid classes (triglycerides

vs. cholesterol) in larval American (clawed) lobsters (Harding and Fraser, 1999), the refractive index of haemolymph and weight to carapace length ratio in juvenile *Jasus edwardsii* (Oliver and MacDiarmid, 2001). Several parameters have been investigated to predict male moult increment in *J. lalandii*, such as hepatopancreas- and abdominal muscle size and composition, as well as weight to carapace length ratio (Cockcroft, 1997). Results suggested that aspects of biochemical hepatopancreas composition (moisture, lipids, proteins) are promising candidates.

Mature *J. lalandii* moult and reproduce once a year (Pollock, 1986). Males moult in late spring to early summer (September-December), well before females in late austral autumn to early winter (April-June) and the mating period in winter (July-August)(Heydorn, 1969a). In males, energy accumulation takes place between the annual moulting events and relies on sufficient prey (Cockcroft, 1997). They use 99% of energy reserves accumulated during intermoult for growth, in contrast to females which use 5% (Zoutendyk, 1990). While the maturing ovaries of females become a second centre of energy accumulation (Harrison, 1990), large energy reserves in males are stored in the hepatopancreas or midgut gland (Cockcroft, 1997). Due to its large relative size (~30% of body weight), though, the storage capacity of the abdominal muscle ("tail") is also substantial (Smith et al., 2004). However, metabolite concentrations in the latter are seasonally less changeable than in the hepatopancreas (Cockcroft, 1997). Main energy metabolites are lipids and proteins but although proteins were previously regarded as the principal energy source in crustaceans (New, 1976), more recent research revealed that crustaceans differ widely in this regard and that lipids play a major role (Sánchez-Paz et al., 2006). Carbohydrate reserves (in the form of glycogen) play a minor role: they are limited in crustaceans and only power short-term high-intensity anaerobic muscle work, such as tail-flip, after phosphagens are used up (England and Baldwin, 1983). In addition, carbohydrates provide structural chitin building blocks (N-acetylglucosamine) for the formation of the exoskeleton, which are not available for energy production (Charmantier-Daures and Vernet, 2004).

As in other decapods, the hepatopancreas is particularly relevant for many biological processes in palinurids and it is their main organ for lipid digestion and accumulation (Smith et al., 2004; Perera and Simon, 2015; Munian et al., 2021). Its lipid content is therefore high but varies with the moult cycle (Cockcroft, 1997). The lipid deposits in the hepatopancreas serve growth and the formation of the new exoskeleton. Therefore, the peak lipid storage period has a positive correlation to the measured growth increment and was proposed as a potential reliable indicator for growth in males, whereas for proteins, no such relationship was found (Cockcroft, 1997).

A reduced level of lipid accumulation during intermoult by the species was shown to lead to low growth rates and even shrinkage (Cockcroft and Goosen, 1995; Cockcroft, 1997). Since reserve accumulation in male WCRL is almost exclusively aimed at providing energy and structural metabolites for moulting, it is helpful to link these processes to distinct stages in the moult cycle. Moult stage was previously determined by the subjective hardness of the exoskeleton and divided into four "hard shell- and soft-shell states" (Heydorn, 1969a). This is more practical but less exact than analysis of titres of moulting hormone or setagenic analysis (Marco, 2012). Additional information on these processes in *J. lalandii* throughout fishing- and moulting seasons would aid understanding of what determines growth increment at the next moult. This is because the current state of knowledge on direct and indirect environmental factors, such as food availability, that impact growth and reproduction of the WCRL is, as described above, suboptimal for predicting population- and resource development and, in turn, sustainable management. In particular, the impact during the reserve accumulation phase is important, because starvation or sub-optimal food supply may impact biological events (moult, spawning) long before they happen.

Historically, growth rates of WCRL of commercial size differ from area to area (Cockcroft and Goosen, 1995). It was found, for example, that there is a substantial difference between two catch areas in close proximity along the Cape Peninsula (about 30 km apart): Olifantsbos and Hout Bay (Figure 1). In Olifantsbos, growth rates have been substantially lower than in Hout Bay since the early 1990s and the two sites were therefore compared previously (Cockcroft, 1997). Such regional growth differences are assumed to be caused by variations in food availability and composition (Newman and Pollock, 1974; Melville-Smith et al., 1995, Chandrapavan et al., 2009, 2010).

The aims of this study were to determine and compare the seasonal variation in the biological cycles (moult and somatic growth rate) and biochemical composition of male lobsters from a high growth (Hout Bay) and low growth (Olifantsbos) area and to determine the potential for the development of a reliable physiological index to predict male somatic growth.

3.2. Materials and methods

Male rock lobsters (n = 24-30 per sampling) of 65 - 82 mm carapace length (CL) were sampled more or less monthly, depending on availability of ship's time and sea conditions, at two sites on the South African west coast (Fig. 1) from March 2010 to March 2012, covering two moult cycles. The Hout Bay site ("Die Knol"; 34º04'S, 18º20'E), located in a rock lobster sanctuary, is historically a fast growth area, whereas the Olifantsbos site (34°16'S, 18°22'E), in a commercially exploited area, is characterized by slow lobster growth rates (Cockcroft, 1997). The official fishing season in both areas spans from mid-November to July. Lobsters were sampled from the research vessel RV "Ellen Khuzwayo" with the aid of standard commercial gear (Pollock, 1986) at depths of approximately 80 to 200 m. After capture, total wet weight (w_T) and carapace length (CL) were measured; w_T was determined by digital balance to the nearest g, whereas Vernier calipers were used to measure CL (from the tip of the rostrum to midpoint of posterior edge of carapace) to the nearest 0.1 mm. Animals were subsequently sacrificed and dissected. Abdominal muscle (tail) wet weight (w_A) was determined after dissection, whereas hepatopancreas was transferred into pre-weighed vessels. Samples were then blast-frozen aboard immediately and later transported to freezers at the laboratory where they were kept at -40 °C for later weight determination and biochemical analysis (Hout Bay samples only). These samples remained sealed from air in a plastic vial to prevent auto-oxidation of the lipids in the sample. For determination of moult stage, a pleopod was sampled from some batches of Hout Bay lobsters, which were individually placed in an Eppendorf tube for storage at -25 °C.

Growth or moult increment (increase in lobster CL at moult) in each sampling area was determined from tagged- and recaptured animals, according to Goosen and Cockcroft (1995), during routine research cruises. Values were taken for the fishing seasons spanning from 2008-2009 to 2013-2014 so that they provide the context for the period of biological and biochemical sampling (described above).

Biochemical analyses of hepatopancreas samples were limited to Hout Bay lobsters to minimize cost and labour. These analyses were performed by the Council of Scientific and Industrial Research (CSIR) of South Africa as follows: After removal from the freezer, hepatopancreas weight was determined, the sample defrosted and homogenized using a Ultraturrax blender (IKA, Germany). Subsequently, moisture content (by heating for 24 hours at 104 °C), crude protein (LECO protein analyzer, measurement of nitrogen by standard

DUMAS; % nitrogen was converted to % protein using the factor 6.25) and total lipid content (Bligh and Dyer, 1959) were determined from aliquots. Ash and carbohydrate contents were determined by difference (% Carbohydrates and Ash = 100 % - sum of (% lipids + % moisture + % protein).

Somatic indices, i.e. hepatosomatic index (HSI) and abdominal muscle index (TI) were calculated according to equations:

$$I. \quad HSI = \frac{Hepatopancreas\ wet\ weight}{Total\ wet\ weight} \times 100\%$$

$$II. \quad TI = \frac{Tail\ wet\ weight}{Total\ wet\ weight} \times 100\%$$

The stage of individual Hout Bay lobsters in the moult cycle was determined via microscopic analysis of setagenic features of pleopods as outlined in Marco (2012) for adult *J. lalandii*. Briefly, the distal section of the pleopods were floated on a cavity slide, covered with a cover slide and examined with a compound eclipse Ni microscope (Nikon, Japan). The moult stages of *Jasus lalandii* are divided into stages AB (post-moult), C (intermoult), D_0 - $D_{3\text{-}4}$ (pre-moult)(Marco, 2012). Substages of premoult stage D_1, such as D_1', D_1", were lumped together for simplicity and all moult stages were later coded as numerical values for analytical purposes (C = 1, D_0 = 2, D_1 = 2.5). This analysis was also limited to Hout Bay lobsters to minimize labour.

Statistical differences in biological and biochemical parameters and linear regression statistics were analyzed by one-way ANOVA followed by the appropriate parametric- or nonparametric post-hoc test (given in figure captions). All statistics were calculated by using Sigma Plot version 14.0. The significance level chosen throughout was $p < 0.05$.

3.3. Results

Growth increment

Except in the 2009-2010 season, when it was almost equal, mean moult increments (Table 1) measured in Hout Bay were substantially larger than those in the Olifantsbos area. The lowest value in Hout Bay of 2.5 mm in 2009-2010 was close to the highest value in Olifantsbos in all years analysed. Due to unavailability of sea time, there was no growth rate measurement in Hout Bay in the 2012-2013 season.

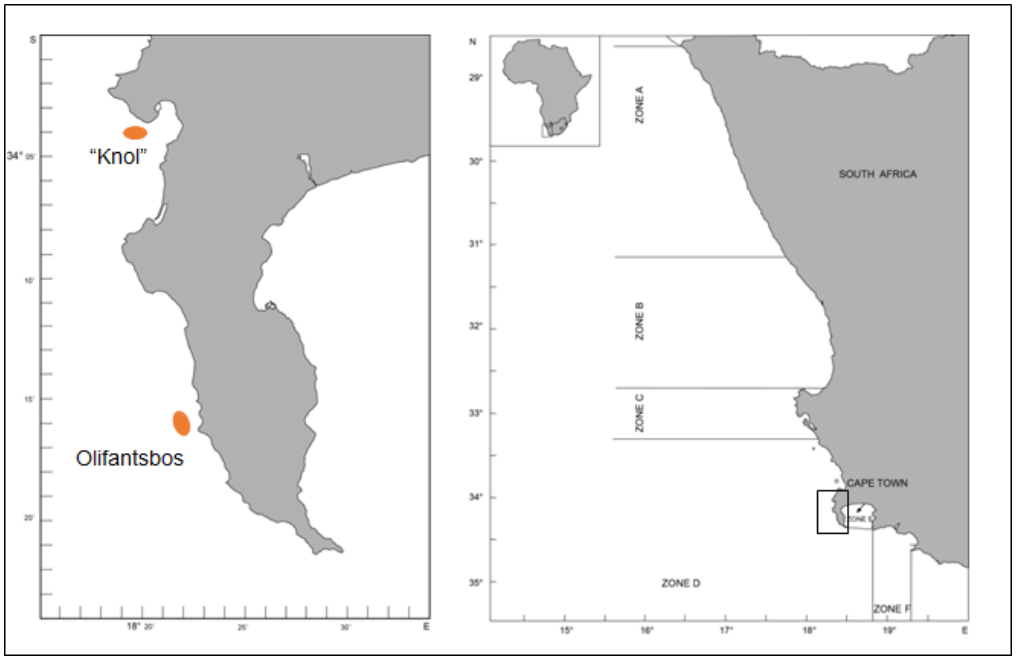

Figure 1. Study area, showing the two sampling sites, i.e. the fast-growth area "Knol" (Hout Bay) and the slow-growth area Olifantsbos (orange).

Biological data

Due to the targeted size class of lobsters, variation in average total wet weight (w_T) during the research period was low (Table 2). Lobsters from the Hout Bay sampling site ranged from 188 – 243 g in mean total weight (CL 70 – 76 mm) whereas in Olifantsbos total weight averaged 196 – 227 g (CL 71 – 75 mm).

Table 1. Mean moult increments (mm) measured in the Hout Bay and Olifantsbos areas during 2008-2014 from tagged- and recaptured animals.

Fishing season	n	Hout Bay	n	Olifantsbos	% difference
2008-2009	309	2.2 ± 1.9	283	1.6 ± 1.4	27*
2009-2010	383	2.5 ± 1.8	510	2.3 ± 1.5	7
2010-2011	34	2.7 ± 1.2	596	1.6 ± 1.3	41*
2011-2012	39	3.4 ± 1.9	37	1.5 ± 1.1	55*
2012-2013	n.a.	2.8[#]	67	1.8 ± 1.8	34[#]
2013-2014	33	3.4 ± 1.1	447	2.0 ± 1.7	40*

Values are given as mean ± SD. *Significantly different between areas in the same fishing season ($p<0.05$, t-test). [#]No value determined. Estimated from average differences in growth increment of all other fishing seasons. No tag returns due to unavailability of own ships and no fishing in the area by industry.

Mean tail weight w_A ranged from 64 g in February 2011 to 80 g in December 2010 in Hout Bay and from 64 g in July 2010 to 74 g in February 2012 in Olifantsbos (Table 2). Average TI ranged from 32% of w_T in July 2010 and November 2011 to 34% in several months of each year in Hout Bay (Table 2, Fig 2A). In Olifantsbos it ranged from 32% to 34%, each in several months of each year (Table 2, Fig. 3A). There was no clear seasonal trend in either area. The mean tail weight : carapace length (w_A/CL) ratio in Hout Bay ranged from 0.9-1.1 throughout the sampling period (Table 2, Fig. 2B), although values were higher in each summer. Similarly, in Olifantsbos the range was 0.9–1.0 (rounded, Table 2, Fig 3B). In general, the w_A/CL ratio is lower in Olifantsbos and never reached a value of 1. There was no trend in the Olifantsbos data. In both areas, the mean hepatopancreas wet weight (w_H) was highest over the austral summer months after which it declined to reach a minimum in autumn/early winter (Table 2). In Hout Bay, it fluctuated between 8.6 g in October 2010 to 12.3 g in December 2010. Similarly, in Olifantsbos, w_H ranged from 9.0 g in November 2011 and 11.7 g in February 2012.

In Hout Bay, mean Hepatosomatic Index (HSI) ranged from 4.3 in October 2010 to 5.4 % of w_T in January 2011. It was generally high around December to April each year and lowest several months later (Table 2, Fig. 2C). Lowest values occurred just before and after moult before increasing to peak values between January and May. In Olifantsbos, the general trend was similar with the lowest value of 4.4 % measured in October 2010 and the highest of 5.3 % in May 2011 (Table 2, Fig. 3C).

Moult cycle

The stage in the moult cycle was determined from some batches (months) of lobsters in the Hout Bay area. All analyzed animals were either in intermoult stage C and premoult stages D_0 and D_1. (Fig. 4A). Stage D_1 consists of three sub-stages. For simplicity and given the small sample size, D_1 was not further divided and all sub-stages are summarized as D_1.

Lobsters in stage C were present in all months sampled and numbers ranged from 4 to 26 with the highest number in March 2010 (26, 87%) while the lowest was found in October 2011 (4, 16%). It was also the dominant moult stage in animals caught in January (15, 60%) and February (16, 64%) 2011. The number of lobsters in stage D_0 ranged from 4 to 16 (16-64%) and was present throughout each months' samples. The share of the stage was only low in March (4, 13%) and July 2010 (9, 30%) and February 2011 (9, 36%); in all other months, it reached 40 – 64%.

Table 2. Biological data of male *J. lalandi* caught at two sites from March 2010 to March 2012.

Date	n	CL mm	w_T g	w_A g	w_H g	TI % w_T	w_A/CL	HSI % w_T	Moult numerical
Hout Bay									
Mar 2010	30	75 ± 3	228 ± 29	75 ± 8	11.3 ± 2.2	33 ± 2	1.0 ± 0.1	4.9 ± 0.6	1.1 ± 0.3
Apr 2010	30	76 ± 3	243 ± 31	79 ± 10	11.9 ± 2.0	33 ± 2	1.0 ± 0.1	4.9 ± 0.6	n.d.
May 2010	30	73 ± 2	210 ± 17	69 ± 5	10.9 ± 1.4	33 ± 1	0.9 ± 0.1	5.2 ± 0.6	n.d.
Jul 2010	30	72 ± 2	201 ± 19	65 ± 6	8.8 ± 1.1	32 ± 2	0.9 ± 0.1	4.4 ± 0.5	1.8 ± 0.7
Oct 2010	24	72 ± 2	199 ± 23	68 ± 7	8.6 ± 1.4	34 ± 2	0.9 ± 0.1	4.3 ± 0.6	1.7 ± 0.6
Nov 2010	25	73 ± 2	216 ± 19	72 ± 6	9.5 ± 1.9	34 ± 2	1.0 ± 0.1	4.4 ± 0.7	1.8 ± 0.5
Dec 2010	25	75 ± 4	237 ± 39	80 ± 13	12.3 ± 2.5	34 ± 2	1.1 ± 0.1	5.2 ± 0.6	1.6 ± 0.5
Jan 2011	25	73 ± 3	215 ± 23	73 ± 7	11.7 ± 1.3	34 ± 2	1.0 ± 0.1	5.4 ± 0.5	1.4 ± 0.5
Feb 2011	25	70 ± 4	188 ± 32	64 ± 10	9.9 ± 2.5	34 ± 2	0.9 ± 0.1	5.2 ± 0.7	1.3 ± 0.5
Apr 2011	25	71 ± 3	199 ± 23	68 ± 7	9.7 ± 1.7	34 ± 2	1.0 ± 0.1	4.8 ± 0.5	n.d.
Jul 2011	25	73 ± 2	210 ± 19	69 ± 6	10.3 ± 1.7	33 ± 2	1.0 ± 0.1	4.3 ± 0.8	1.2 ± 0.4
Oct 2011	25	72 ± 3	203 ± 21	69 ± 6	8.6 ± 1.7	34 ± 2	1.0 ± 0.1	4.3 ± 1.1	1.9 ± 0.5
Nov 2011	25	73 ± 2	206 ± 16	65 ± 4	9.5 ± 1.2	32 ± 1	0.9 ± 0.0	4.6 ± 0.6	n.d.
Dec 2011	23	75 ± 7	241 ± 70	79 ± 22	11.2 ± 2.3	33 ± 1	1.0 ± 0.2	4.8 ± 0.9	n.d.
Jan 2012	25	75 ± 3	228 ± 29	78 ± 11	12.0 ± 2.1	34 ± 2	1.0 ± 0.1	5.2 ± 0.5	n.d.
Feb 2012	25	75 ± 3	223 ± 23	73 ± 6	11.2 ± 1.5	33 ± 2	1.0 ± 0.1	5.0 ± 0.6	n.d.
Mar 2012	25	75 ± 3	228 ± 29	78 ± 11	12.2 ± 2.2	34 ± 2	1.0 ± 0.1	5.3 ± 0.5	1.7 ± 0.5
Olifantsbos									
Mar 2010	30	72 ± 2	207 ± 24	71 ± 8	10.1 ± 2.1	34 ± 1	1.0 ± 0.1	4.8 ± 0.8	n.d.
Apr 2010	30	71 ± 2	196 ± 23	66 ± 7	9.2 ± 1.7	34 ± 2	0.9 ± 0.1	4.8 ± 0.7	n.d.
May 2010	30	74 ± 2	219 ± 20	73 ± 5	11.1 ± 1.7	33 ± 1	1.0 ± 0.0	5.0 ± 0.5	n.d.
Jul 2010	30	71 ± 2	199 ± 19	64 ± 6	9.6 ± 1.0	32 ± 1	0.9 ± 0.1	4.5 ± 0.5	n.d.
Oct 2010	30	74 ± 2	215 ± 20	72 ± 6	9.4 ± 1.7	33 ± 2	1.0 ± 0.1	4.4 ± 0.6	n.d.
Nov 2010	30	72 ± 2	214 ± 21	71 ± 6	9.8 ± 1.8	33 ± 2	1.0 ± 0.1	4.6 ± 0.8	n.d.
Dec 2010	30	72 ± 1	203 ± 15	65 ± 5	9.3 ± 1.3	32 ± 2	0.9 ± 0.1	4.9 ± 0.6	n.d.
Jan 2011	30	71 ± 2	213 ± 17	68 ± 7	10.1 ± 1.8	32 ± 2	0.9 ± 0.1	5.1 ± 0.6	n.d.
Feb 2011	30	72 ± 2	198 ± 23	66 ± 7	9.9 ± 2.1	34 ± 2	0.9 ± 0.1	5.1 ± 0.6	n.d.
Apr 2011	30	72 ± 3	208 ± 22	66 ± 7	10.2 ± 1.8	32 ± 2	0.9 ± 0.1	4.9 ± 0.7	n.d.
May 2011	30	72 ± 2	205 ± 14	67 ± 5	11.6 ± 1.5	33 ± 2	0.9 ± 0.1	5.3 ± 0.5	n.d.
Jul 2011	30	72 ± 2	206 ± 20	67 ± 6	11.3 ± 2.2	33 ± 2	0.9 ± 0.1	4.8 ± 0.6	n.d.
Oct 2011	30	72 ± 2	203 ± 19	69 ± 7	10.3 ± 1.5	34 ± 2	1.0 ± 0.1	4.7 ± 0.5	n.d.
Nov 2011	30	72 ± 2	197 ± 21	67 ± 7	9.0 ± 1.3	34 ± 2	0.9 ± 0.1	4.6 ± 0.6	n.d.
Dec 2011	30	72 ± 2	204 ± 17	66 ± 5	10.4 ± 1.6	32 ± 1	0.9 ± 0.0	4.9 ± 0.6	n.d.
Jan 2012	30	73 ± 2	204 ± 16	65 ± 5	11.0 ± 2.3	32 ± 2	0.9 ± 0.0	5.0± 1.0	n.d.
Feb 2012	30	75 ± 2	227 ± 21	74 ± 5	11.7 ± 1.8	33 ± 1	1.0 ± 0.0	5.2 ± 0.7	n.d.
Mar 2012	30	73 ± 2	210 ± 21	67 ± 6	10.4 ± 2.3	32 ± 2	0.9 ± 0.1	5.0 ± 1.0	n.d.

Values are given as mean ± SD. n.d. = not determined. Abbreviations: CL = carapace length, w_T = total wet weight, w_A = tail wet weight and w_H = hepatopancreas wet weight, TI = Tail Index, HSI = Hepatosomatic Index.

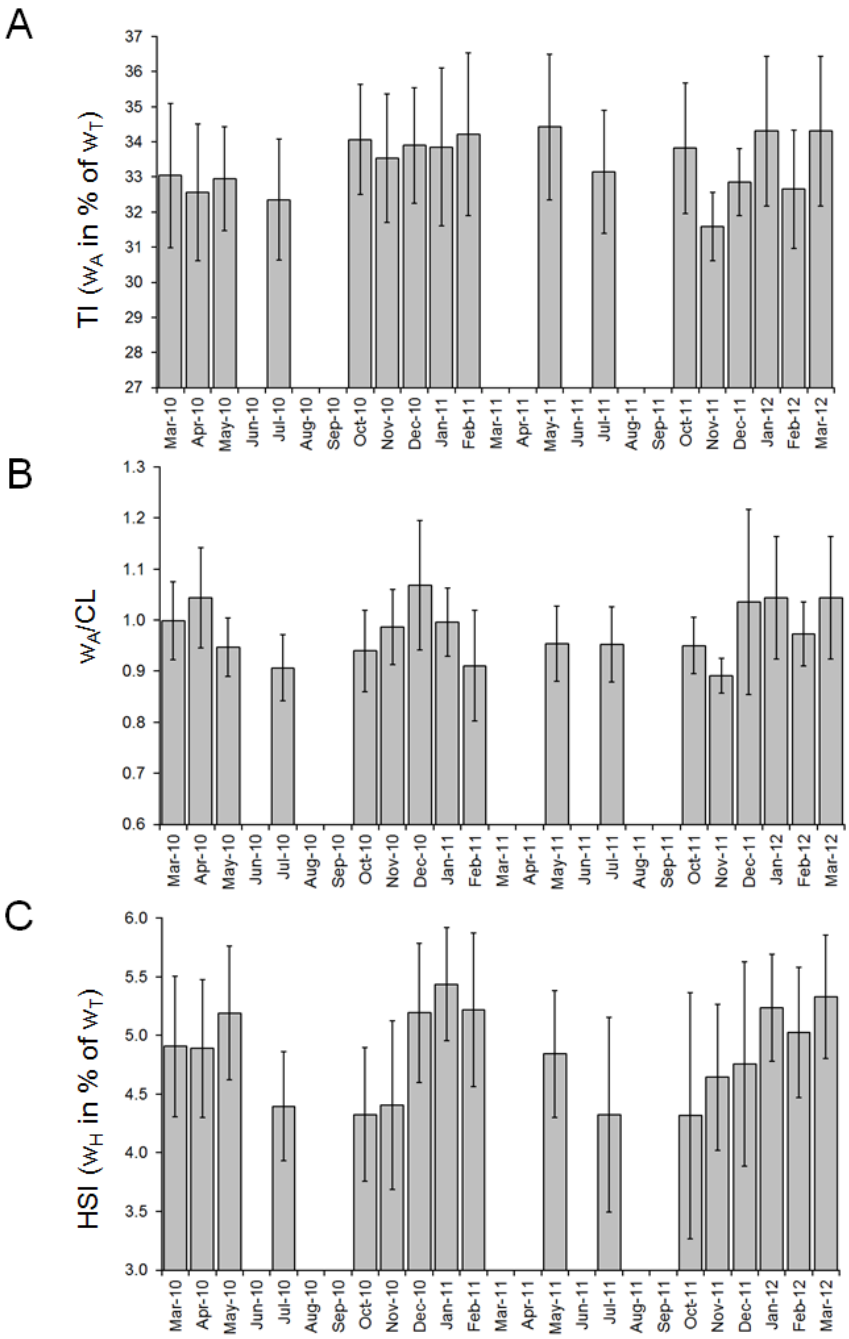

Figure 2. The course of biological parameters from male *J. lalandii* caught at the Hout Bay sampling site (”Knol”) during the period from March 2010 to March 2012. A) Tail Index (TI), B) ratio of tail weight and Carapace length (w_A/CL) and C) Hepatosomatic Index (HSI). Values are means ± S.D., n = 23-30. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

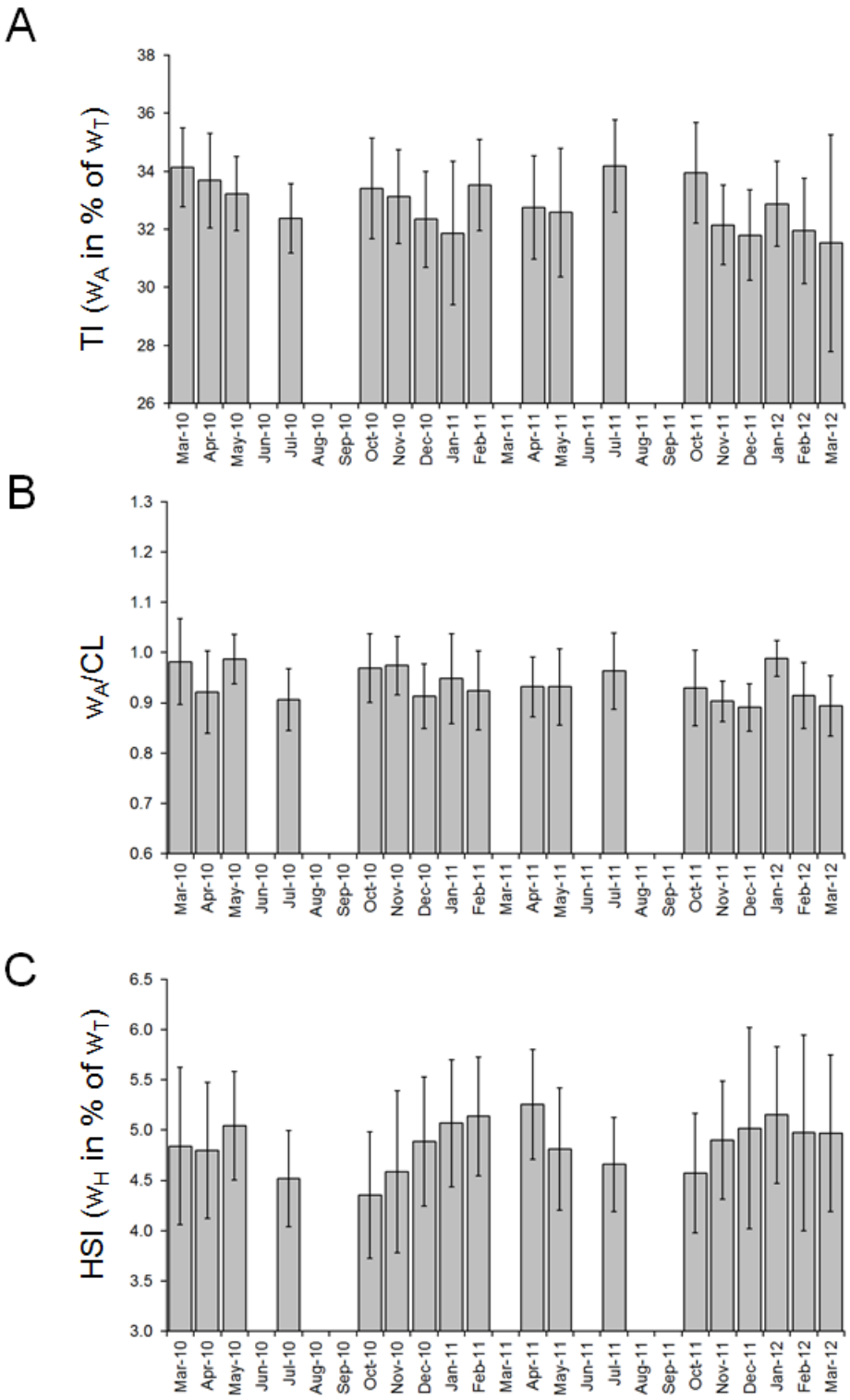

Figure 3. The course of biological parameters from male *J. lalandii* caught at the Olifantsbos sampling site during the period from March 2010 to March 2012. A) TI, B) w_T/CL and C) HSI. Values are means ± S.D., n = 30. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

The number of lobsters in stage D_1 ranged from 1 to 11 (4-37%) and was never a dominant stage in any of the months sampled. D_1 was only observed in 4 of the 10 months sampled. The highest share was recorded in July 2010 (11, 37%) and October 2011 (7, 28%).

To achieve a clearer trend by possibly reducing yearly variation, moult stage numbers were accumulated, where possible, for the same months of the whole sampling period (Fig. 4B). These accumulated results show that lobsters caught throughout the year are mainly in stages C and D_0 (80 – 100%) and that only before the moulting season, animals in D_1 occurred in relevant numbers (8 – 20%). Early in the year (February – April), most lobsters (60-64%) were in intermoult stage. Thereafter, this percentage declines to below 30% into November and increases again towards end of the year (44%). The share of lobsters in stage D_0 increased from 34-40% early in the year to a maximum of 64% in November. In December, their share is lower again at 56%. To present the above data in a simple, continuous trend, moult stages were coded and converted to numerical values (Table 2, Fig 4C). Early in the moult cycle (stage C), values would be low and increase as the cycle progresses. The lowest value of 1.1 was recorded in March 2010 and the highest of 1.9 in October 2011. Lowest values were usually recorded early each year and highest in the second half of the year.

Hepatopancreas biochemistry

Moisture content: The mean moisture content of the hepatopancreas is inverse to w_D (Table 3) and is therefore not explicitly reported here. It serves, however, as reference value for the content of other compounds and is used to determine the actual grams of lipid and protein in the hepatopancreas on a dry weight basis. Moisture content ranged from 63.7% in January 2012 to 75.5% in October 2010. Highest moisture contents were recorded in summer, i.e. before moulting after which they declined until winter (Table 3; Fig. 5A).

Lipid content: Lipid content (per w_D) was lowest in October to December, followed by an increase to peak values during March to July and a subsequent decline (Table 3; Fig. 5B). Peak values were in the range of 48 – 52% and lowest values in the range of 27 – 36%.

Protein content: Protein content (per w_D) follows a similar trend as moisture content, both of which are inverse to that of lipid content (Fig. 5). Lowest values of approximately 40% were recorded in autumn whereas peak values of about 50 – 55% occurred from October to December.

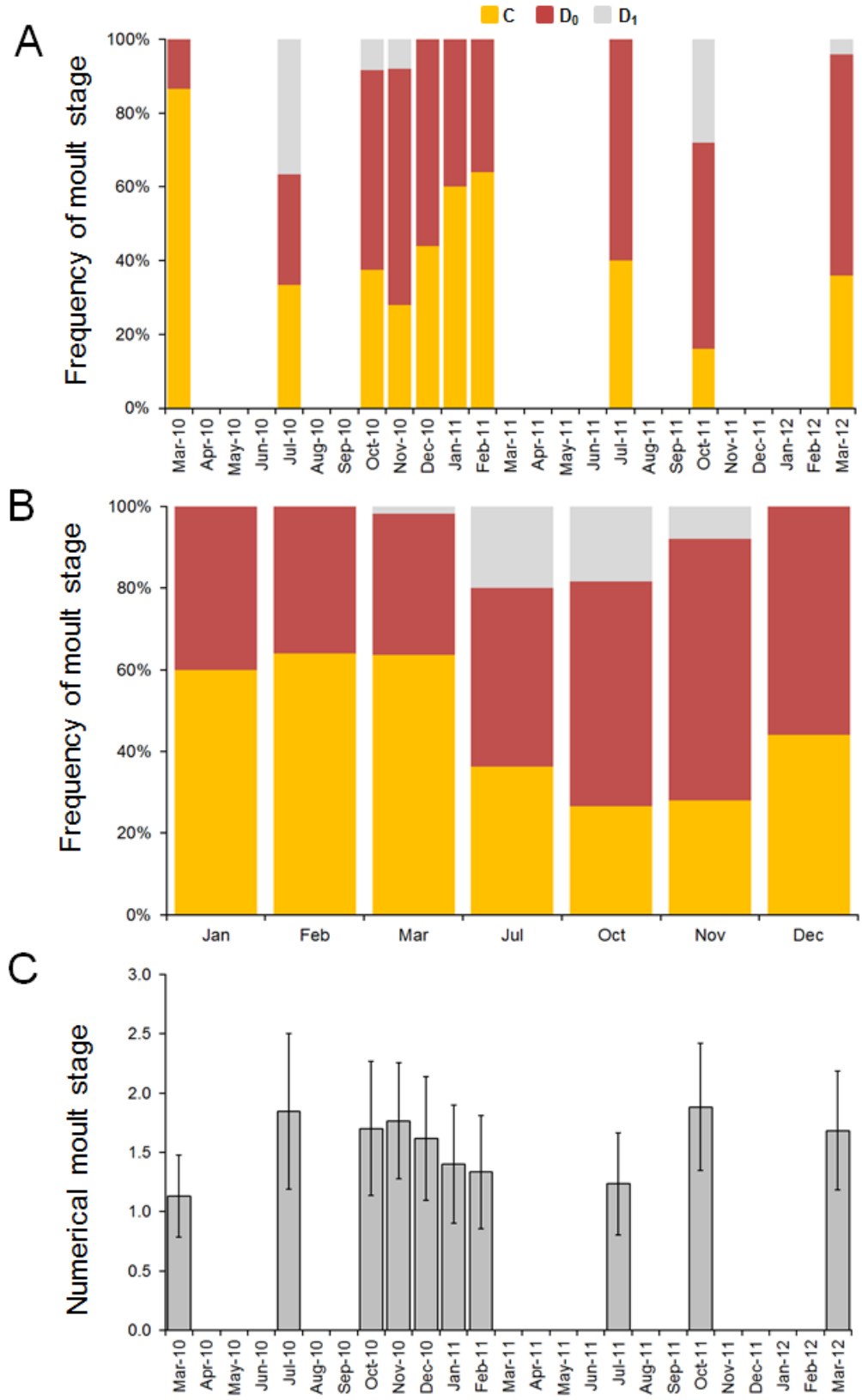

Figure 4. Moult stage of male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during select months from March 2010 to March 2012. A) relative frequency of moult stage distribution, B) accumulated moult stage frequency for same months of all sampled years and C) numerical course of stage in moult cycle throughout the months of the sampling period (coded C = 1, D_0 = 2, D_1 = 2.5). In C, values are means ± S.D., n = 24-30.

Carbohydrate and ash content: The course of these compounds (per w_D) seemed to follow the trend of total proteins but much less pronounced (Fig. 5B). Lowest values of approximately 8 – 12% occurred May to July whereas highest values of about 15 - 18% were recorded in late spring/early summer (Table 3 Fig. 5B).

Table 3. Biochemical composition of hepatopancreas from male *J. lalandi* caught at the Hout Bay sampling site ("Die Knol") from March 2010 to March 2012.

Date	n	moisture	w_D	Total Lipids	Proteins	Carbohydrates and ash
		% w_W	% w_W	% w_D	% w_D	% w_D
Mar 2010	30	67.7 ± 5.2	32.3 ± 5.2	41.8 ± 10.5	43.9 ± 7.6	14.3 ± 5.3
Apr 2010	30	67.6 ± 5.2	32.4 ± 5.2	41.4 ± 9.9	43.9 ± 7.8	14.7 ± 5.0
May 2010	30	68.1 ± 5.2	31.9 ± 5.2	44.0 ± 10.4	41.9 ± 6.4	14.1 ± 5.9
Jul 2010	30	69.0 ± 5.4	31.0 ± 5.4	48.9 ± 10.4	39.9 ± 6.6	11.2 ± 6.6
Oct 2010	24	75.5 ± 6.3	24.5 ± 6.3	33.2 ± 6.5	50.3 ± 4.0	16.5 ± 7.0
Nov 2010	25	73.2 ± 7.0	26.8 ± 7.0	36.4 ± 11.0	51.9 ± 7.2	11.6 ± 5.3
Dec 2010	25	73.3 ± 4.5	26.7 ± 4.5	35.3 ± 14.6	49.9 ± 8.2	14.8 ± 9.3
Jan 2011	25	70.0 ± 8.1	30.0 ± 8.1	37.3 ± 11.4	47.0 ± 5.2	15.7 ± 8.1
Feb 2011	25	66.0 ± 4.2	34.0 ± 4.2	41.0 ± 13.7	44.9 ± 7.7	14.1 ± 6.6
May 2011	25	67.1 ± 4.3	32.9 ± 4.3	48.5 ± 10.8	41.9 ± 6.9	9.6 ± 5.0
Jul 2011	25	68.0 ± 2.4	32.0 ± 2.4	51.6 ± 14.5	39.9 ± 9.1	8.5 ± 6.9
Oct 2011	25	71.7 ± 4.5	28.3 ± 4.5	34.0 ± 8.8	50.0 ± 4.7	16.0 ± 7.0
Nov 2011	25	69.0 ± 5.8	31.0 ± 5.8	26.8 ± 6.3	55.1 ± 6.2	18.1 ± 6.9
Dec 2011	25	65.6 ± 6.0	34.4 ± 6.0	35.9 ± 10.8	50.0 ± 6.6	14.2 ± 8.1
Jan 2012	25	63.7 ± 5.9	36.3 ± 5.9	40.1 ± 8.0	46.0 ± 6.3	14.0 ± 9.3
Feb 2012	25	66.6 ± 3.1	33.4 ± 3.1	41.3 ± 9.2	44.9 ± 5.5	13.8 ± 7.0
Mar 2012	25	65.2 ± 4.0	34.8 ± 4.0	43.2 ± 10.9	42.3 ± 5.4	14.5 ± 6.8

Values are given as mean ± SD. n.d. = not determined. Abbreviations: w_W = wet weight, w_D = dry weight.

Potential indicators

The biological indicators HSI and TI were analyzed per moult stage for the Hout Bay sampling site, for individual animals whose moult stage had been determined (see above). For this, values of the same stage from the entire sampling period were pooled, i.e. all intermoult stage C (n = 117) and premoult stages D_0 (n = 117) and D_1 (n = 25). Their respective HSI and TI were then compared (Fig. 6). HSI values were very similar in all three moult stages ~5% and only D_1 was ~8% lower than the two other stages (not significant) at 4.6% (Fig. 6A). TI was highest (33.7%) in intermoult (stage C) and declined slightly during premoult stages D_0 (33.5%) and D_1 (32.9%, not significant; Fig. 6B). There is a declining trend in TI from stage C to stage D_1 from 33.7 to 32.9% (-2.4%) although these differences were not significant. Hepatopancreas lipid content was highest in stage C (13.2%) and at a 11% lower level (~11.7% of w_D) at stages D_0 and D_1,

respectively (Fig 6C). The differences were not significant. Protein content was at a very similar level of approximately 13% at each stage (Fig. 6D).

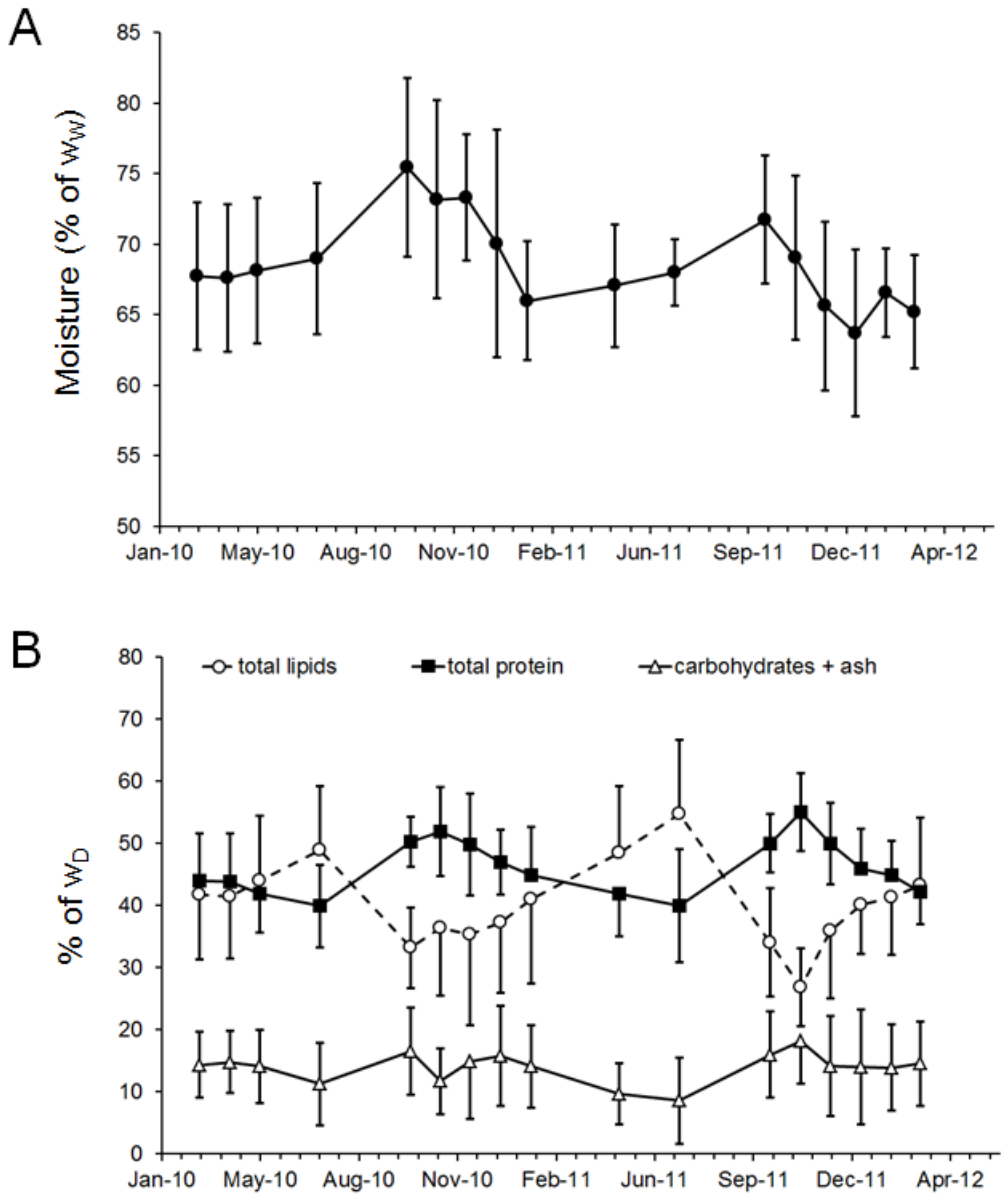

Figure 5. The course of the biochemical composition of hepatopancreas samples from male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. A) Moisture content, B) lipid-, protein- and carbohydrate and ash contents. Values are means ± S.D., n = 24-30. Abbreviations: w_W = wet weight, w_D = dry weight.

Based on maximum HSI, "peak accumulation" months were identified (Cockcroft, 1997). From this period of each year (2010: Mar, Apr, May; 2011: Jan, Feb, Apr; 2012: Jan, Feb, Mar), certain biological and biochemical parameters were accumulated and analysed together. Whereas HSI was in a narrow range of about 5.0 - 5.5% in Hout Bay, values for 2011 and 2012 were significantly higher than that for 2010 (Fig. 7A). In Olifantsbos, values ranged from 4.9 to 5.2% and the value for 2012 was significantly higher than in the preceding years (Fig. 7E). TI was similar in each peak period in Hout Bay at 33 - 34% (Fig. 7B). Despite this narrow range, values for 2011 and 2012 were significantly higher than in 2010. TI declined significantly from 34 to 30% in Olifantsbos from 2010 to 2012 (Fig. 7F). Lipid content in Hout Bay was highest in 2010 (17.2%) and at a level of about 12% in both of the following years (Fig. 7C). Protein was lower at around 13% in both 2010 and 2011 than the 15.3% in 2012 (Fig. 7D).

Analysis of interrelationships of hepatopancreas parameters of all animals sampled at the Hout Bay site revealed that there is a decrease in moisture content as hepatopancreas size increases (Fig. 8A). Lipid- and protein concentrations showed increasing and decreasing trends, respectively, as hepatopancreas size increases, although with shallow slopes (Fig. 8B,C). These inverse trends are also visible in the significant correlation of both compounds in Fig. 8D.

Table 4. Annual differences in select biological and biochemical data from July 2010 and July 2011 from the Hout Bay fishing area.

	n	Growth increment	w_T	W_H	HSI	Lipid dry weight	Lipid wet	Lipid per hepatopancreas
		mm	g	g	% w_T	% w_D	% w_W	g
July 2010	30	2.7 ± 1.2	201 ± 19	8.8 ± 1.1	4.4 ± 0.4	48.9 ± 10.4	15.1 ± 4.2	1.3 ± 0.4
July 2011	25	3.4 ± 1.8	210 ± 19	10.1 ± 2.0	4.6 ± 0.5	51.6 ± 14.5	17.3 ± 3.5	1.7 ± 0.4
difference		26%*	4%	9%	6%*	12%*	9%*	24%*

Values are given as mean ± SD. Abbreviations: w_T = total wet weight, w_W = wet weight. w_D = dry weight, w_H = hepatopancreas wet weight, HSI = Hepatosomatic Index. *Significantly different between July 2010 and July 2011 ($p<0.05$, t-test).

Lobsters were, with few exceptions, in a narrow size range and hence comparable. Absolute lipid weight per hepatopancreas was therefore used as a factor to estimate the total amount of lipids available for growth. First, a seasonal course was constructed for this parameter (wet weight lipid concentration multiplied by hepatopancreas wet weight): Highest values occurred at the beginning of each calendar year (1.4 – 1.9 g) and lowest (0.8 – 0.9 g) in austral spring (Fig. 9). The highest value in 2011 was observed in July (1.7 g) whereas it was highest (1.6 g)

in March in 2010 (Fig. 9, Table 4). Although lobsters caught in July 2011 where slightly larger, the amount of lipid per hepatopancreas was 24% higher, owing to both the larger hepatopancreas weight and the higher lipid concentration. Although lipid weight per hepatopancreas only peaked in July in 2011, lipid concentration in the hepatopancreas peaked in July of both years (Table 3, Fig. 5B). July was also the last sampling point before moult. Data for July 2010 and 2011 were therefore further analysed for their potential to predict growth in the subsequent moult. In 2010, lipid content was higher both per w_D (+12%) and w_W (+9%) in 2011 than in 2010. In addition, lipid per hepatopancreas was 24% higher in 2011, whereas w_T as well as HSI were only slightly higher (Table 4).

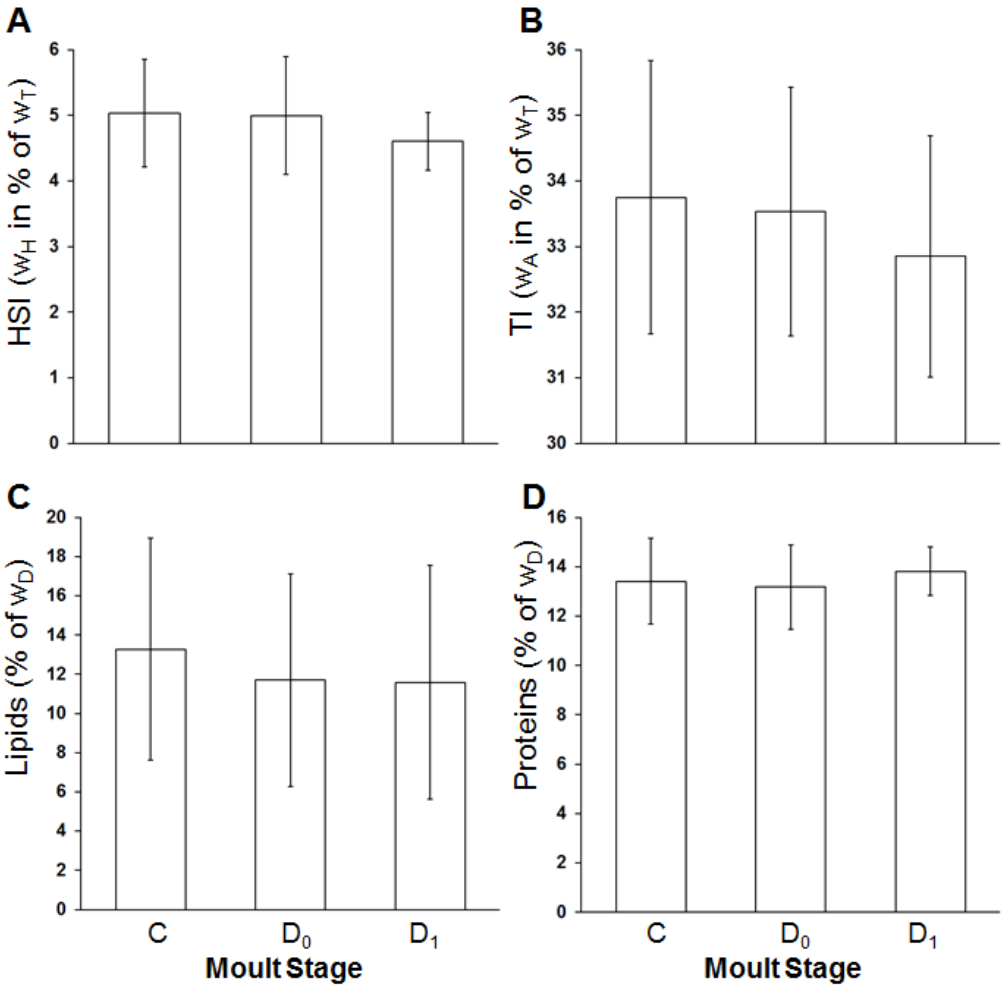

Figure 6. Levels of A) HSI, B) TI, C) hepatopancreas lipid content and D) hepatopancreas protein concentration during the respective moult stage. Analysis was carried out on lobsters from the Hout Bay sampling site only. No significant differences ($p < 0.05$) between moult stages were observed (one-way ANOVA). Values are means ± S.D., n = 117 (stage C), n = 117 (stage D_0), n = 23 (stage D_1). Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_D = dry weight.

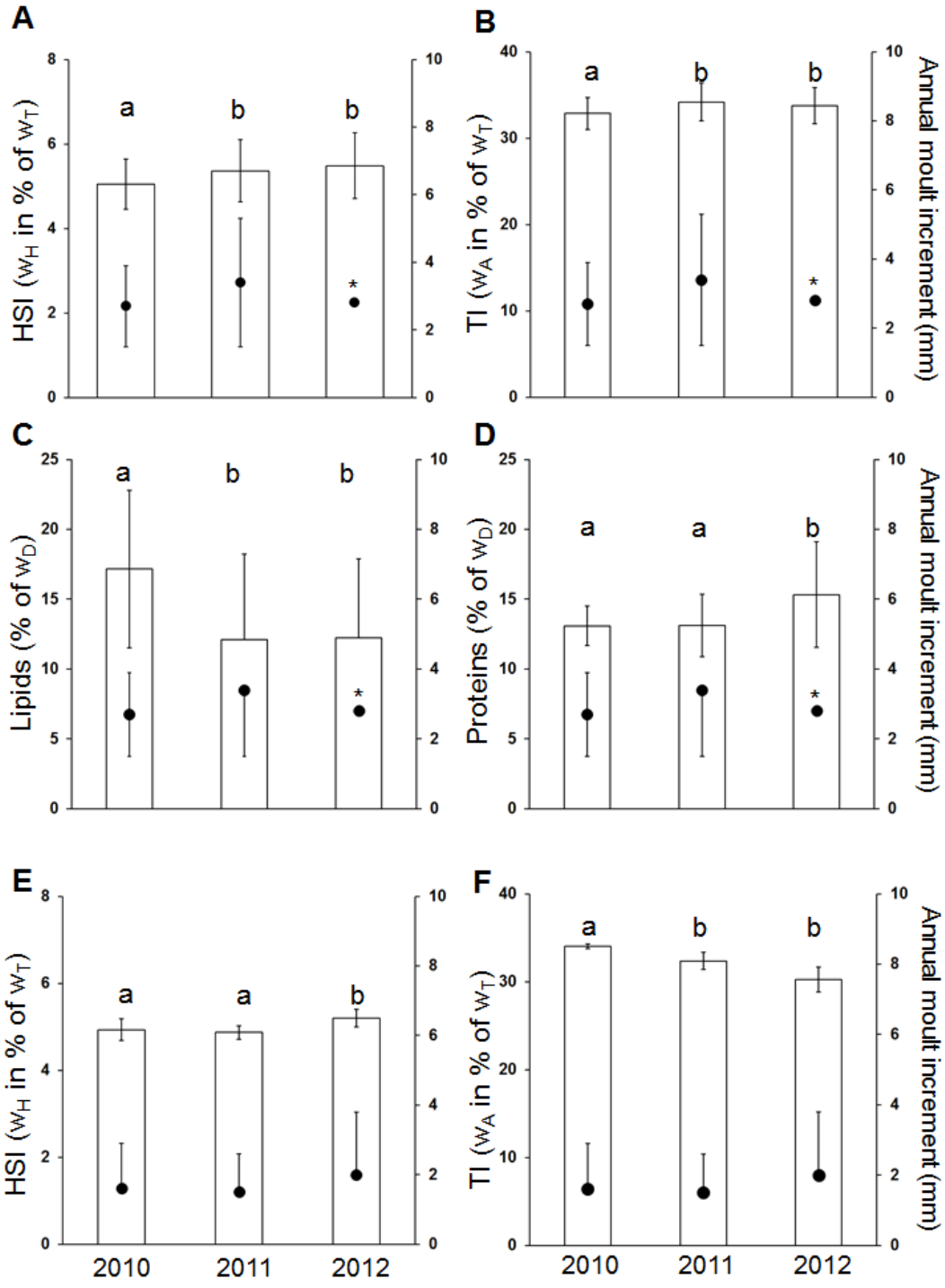

Figure 7. Biological and biochemical data (bars, left y-axis) from male *J. lalandii* caught at both sampling sites during the respective annual peak accumulation periods from March 2010 to March 2012. A) HSI, B) TI, C) lipid content and D) protein content from the Hout Bay sampling site. E) HSI and F) TI from the Olifantsbos sampling site. For information, respective annual growth rates are given as full circles (y-axis). Note, no annual growth rate is available for the Hout Bay sampling site for 2012 due to unavailability of ships. *Estimated, see Table 1. Different lower-case letters indicate a significant difference ($p < 0.05$) between years (one-way ANOVA followed by Kruskal-Wallis Analysis). Values are means ± S.D., n = 55-90. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_D = dry weight.

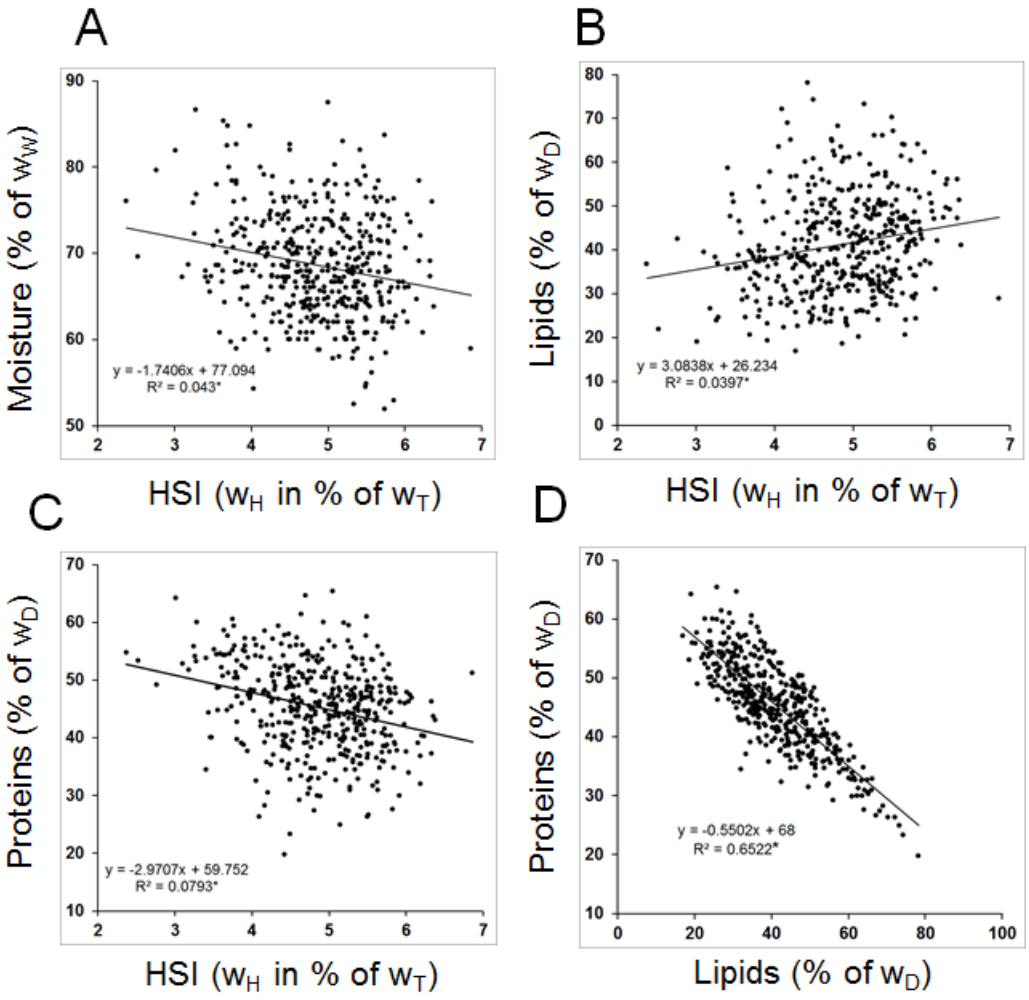

Figure 8. Interrelationships of hepatopancreas A) relative weight (HSI) and moisture content, B) HSI and lipid concentration, C) HSI and protein concentration and D) lipid concentration and protein concentration from all WCRL analysed from the Hout Bay sampling site (n = 442). *Significant correlation ($p<0.05$) of linear regression (ANOVA). Abbreviations: w_W = wet weight, w_D = dry weight, w_T = total wet weight, w_H = hepatopancreas wet weight.

3.4. Discussion

The present research has confirmed some previous findings: Long-standing growth trends and differences between the two sampling sites (Goosen and Cockcroft, 1995; Cockcroft, 1997), that triggered previous studies, persist. Annual growth increment is still consistently higher by more than 1 mm (0.2 – 1.9 mm) at the Hout Bay sampling site than in Olifantsbos (Table 1) over the years evaluated in the present study. The causes of the difference as well as strong inter-annual growth variations are not well understood (Cockcroft, 1997). It was assumed that food availability is the reason for geographic differences but also for annual variations (Newman and Pollock, 1974; Pollock, 1982; Chandrapavan et al., 2009, 2010). Mean growth increment in the present study ranged from 2.2 to 3.4 mm in Hout Bay and 1.5 to 2.3 mm in Olifantsbos. This compares with 2.0 – 4.2 mm and 1.3 – 2.2 mm, respectively, of similar-sized

lobsters in Cockcroft (1997). The latter research was conducted in a period of low growth or even shrinkage along the South African West coast (Melville-Smith et al., 1995; Cockcroft and Goosen, 1995; Pollock et al., 1997). Values are still lower than in the past before the slow growth period (DEFF, 2020), indicating that factors for low growth generally persist. A difference in growth increment of 1 mm of a 200 g lobster seems relatively small. It results, however, in a body weight growth differential of approximately 2.8 g (~1.4%) as calculated according to the conversion of Heydorn (1969b). It can therefore be concluded that (standardized 200 g) lobsters from Hout Bay grew by 2 g more in 2011 than in 2010. This differential growth seems to be area specific since in Olifantsbos, there was no difference between the two years (Table 1).

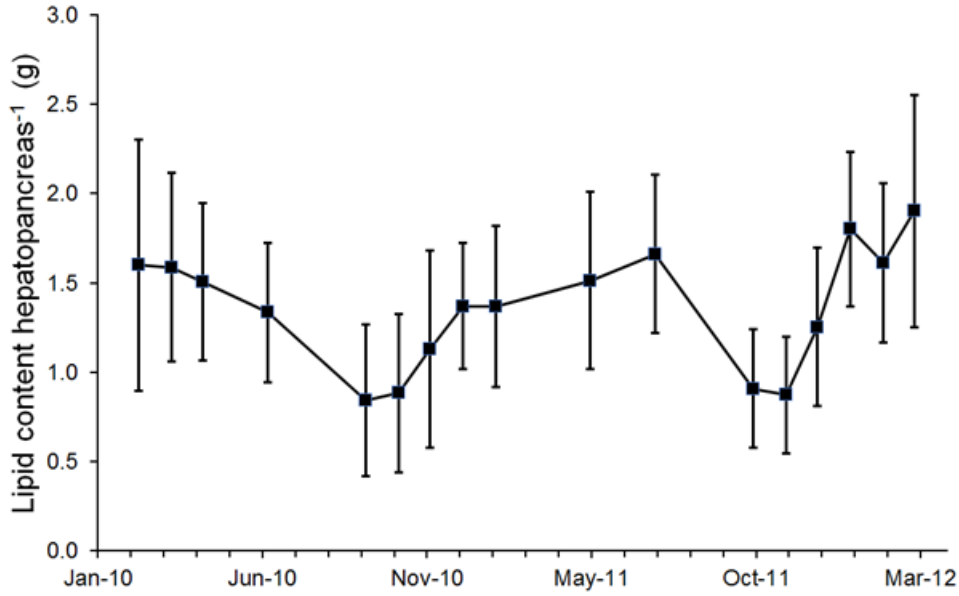

Figure 9. The course of the lipid weight per hepatopancreas of male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. Values are means ± S.D., n = 23-30.

Moreover, seasonal trends in reserve accumulation in the hepatopancreas in the present study are like those found in Cockcroft (1997); an annual increase of HSI until mid to end summer at both sampling sites (Figs. 2,3) and an accumulation of lipids until end of autumn (Fig. 4). The lipid peak did not exactly coincide with the HSI peak and lags several months, results similar to Cockcroft (1997). This is, however, possibly a result of a less defined HSI peak compared with that of lipids in both studies. Lipid content was highest during late intermoult- to early premoult seasons and then decreased rapidly, reaching lowest values immediately before and after moulting. This may indicate their catabolism for energy but also as structural components in processes involved in moult, i.e., muscular- and exoskeleton growth, which start several months before moult. The lipid contents recorded here are at the level known for the palinurid hepatopancreas which can reach 70-75% w_D in the late pre-moult stage and indicate

its prominent role in lipid digestion and accumulation (Cockcroft, 1997; McLeod et al., 2004; Smith et al., 2004). Lipids are particularly relevant for biological processes such as growth (Smith et al., 2004; Perera and Simon, 2015). Furthermore, moisture content and relative protein content follow the same inverse relationship to lipids (Fig. 5,8D) as in the previous study (Cockcroft, 1997): when lipids peak, moisture and relative protein content are lowest (Fig. 5). Water seems to be replaced by the accumulating lipids and proteins as the hepatopancreas grows. Absolute protein levels (not shown) rise at a slower pace than lipids, causing it to decline in relative terms. Based on wet weight, protein levels peak a few months before lipids, similar to Cockcroft (1997). Trends in the tail muscle were less clear which is also similar to the previous study. The tail muscle constitutes a high portion of the total weight of a lobster (~34% with exoskeleton) compared to that of the hepatopancreas of ~5%. In addition, it is the main portion of muscle. Its size and composition may therefore be indicative of physiological condition. In the present study, relative weight of the abdomen (TI, measured as whole tail weight including exoskeleton) from lobsters of the two sites was in a similar range (29 – 34%). Although the abdominal muscle is considered an energy reserve in most crustaceans, it did not appear to be utilized for energy provision here, at least not as judged by organ size. It was previously suggested that abdominal muscle reflects more long-term changes and responds less sensitively to environmental and physiological changes, despite its large relative size (Cockcroft, 1997).

In *J. lalandii* fisheries research, moult stage was previously, if at all, recorded on the basis of shell hardness and stages were subdivided into Hard, Hard Old, Soft New, and Hard New (Heydorn, 1969a). Since this is relatively subjective and needs some experience, the present study used setagenic analysis of pleopods. Results revealed that this method creates some challenges, too (see below). Analysis of moult stage composition of lobsters from the Hout Bay area revealed that there is a large variation and overlap of stages at almost all times of the season (Fig. 4A-C). For example, lobsters in stages C and D_0 were recorded in every month sampled. There may have been a margin of error when differentiating between stages C and D_0 in the present study. These two stages are sometimes difficult to set apart, since no new setae are visible yet in stage D_0 (only from stage D_1"), the gap between old and new epidermis is still very narrow and, in addition, this gap is potentially obscured by pigmentation in adults (Marco, 2012). On the other hand, Marco revealed, based on haemolymph moult hormone titers, that adult *J. lalandii* stay longer in pre-moult stages, especially D_0, than previously estimated from morphological analysis (i.e. setagenic analysis of pleopods). Lobsters do not feed for 15-20

days when they are in stages D_3 to B or early C (Charmantier-Daures and Vernet, 2004; Marco, 2012), therefore, around moulting season, values for such non-feeding lobsters do not appear in the results as they cannot be caught by baited traps. This will, of course, increase the share of lobsters in all other (i.e. feeding) moult stages. As a result, an attempt to capture moulting process numerically is not very conclusive (Fig. 4D). In addition, there was no sampling in August and September in this investigation for logistical reasons. These months, when HSI reaches its minimum (Cockcroft, 1997), could have provided more detail. Nevertheless, moult data provided an additional tool in analyzing biological events during the course of the growth cycle.

Distinct variation of a parameter over the moult cycle or season was deemed essential for consideration as potential growth predictor (Cockcroft, 1997). Two data sets fulfil such seasonality: a) the course of the annual moult cycle and b) the accumulation of metabolic reserves for growth.

In order to test a), biological and biochemical parameters were analysed per moult stage. This, however, did not reveal a clear and useful trend as differences were small and not significant (Fig. 7). Reasons for these inconclusive results may be the low number of D_1 values (n=16-24) compared to the other stages (n=103-119). In addition, stage C is more heterogeneous than the premoult stages (see above), as it is the longest stage (about 130 days, Marco, 2012) and can even be divided into 4 sub-stages by moult hormone titre but not morphologically (Marco, 2012). The latter could not be done in the present study. Accumulation in intermoult takes place over a long period, starting at very low deposition levels. Lipids, for example, accumulate mainly in C_4 to early premoult and rapidly decline from D_2 (Charmantier-Daures and Vernet, 2004; Munian et al., 2021). Levels of accumulation will therefore be very different at the beginning of intermoult period, when accumulation of reserves begins, compared with its end when accumulation reaches its maximum. Unfortunately, an important gap in sampling occurred in August and September when metabolite accumulation is at its lowest. Determination of moult stages seems therefore not a helpful tool for analysis of moult cycle reserve accumulation.

For evaluation of b), the seasonality of “peak accumulation” of reserve levels per year (as described previously in Cockcroft, 1997) were analysed as well as the biochemical dynamics of the hepatopancreas. In terms of “peak accumulation”, the present results do not support the usefulness of certain body parameters, i.e., hepatopancreas moisture content and lipid levels

(as a % of wet weight) at previously identified peak accumulation months, for the prediction of growth in adult male *J. lalandii*. Although the trend regarding hepatopancreas lipid content was clear and seasonal, peak accumulation was not different between years and was not related to growth rates. The hepatopancreas is the central organ for growth in decapods. It serves, amongst other functions, uptake and storage of nutrients, synthesis and catabolism of storage metabolites and provision of material and energy for moult and growth and is the principal organ for lipid metabolism (Gibson and Barker, 1979). Relative size and metabolite content of hepatopancreas are therefore important condition indicators and qualified for potential growth predictors in the previous study (Cockcroft, 1997). Relative weight of hepatopancreas (HSI) from lobsters of the two sites (4.3-5.8%) was in the same range as had previously been reported for palinurids (Cockcroft, 1997; McLeod et al., 2004) and decapods in general (Gibson and Barker, 1979; Whyte et al., 1986; Dall, 1981). There were no differences between the sites despite the lower growth in Olifantsbos. HSI peaked around December to April/May each season, indicating the buildup of reserves for growth and moulting. Peaks are even less sharp than in previous research and the subsequent drop was not as pronounced as in Cockcroft (1997). This was probably a result of the smaller, and maybe insufficient, sample size in the present study. It is perhaps also an indication that biological events have spread more widely and are not as closely synchronized anymore. Females in berry, for example, occurred historically until October (Heydorn, 1969a) but have most recently been found in numbers as late as December (van Zyl, personal communication). We speculated that this may be a result of climate change. Accumulated Hout Bay peak HSI values were lower in 2010 than in the two following years, corresponding with the 2010 and 2011 moult increments. The 2012 moult increment was also higher than in 2010 but was only estimated. The same is true for TI. The w_A/CL ratio showed some seasonality in Hout Bay samples where it peaked around each year end, i.e., shortly after moult (Fig. 2B), whereas no such seasonality was observed in Olifantsbos (Fig. 3B). Cockcroft (1997) had previously not found any seasonality in either of the two sampling sites. This ratio was therefore not considered further as a potential growth predictor. It is noteworthy that w_A/CL ratio is generally lower in Olifantsbos (Table 2) and may therefore be useful in expressing area differences.

In the precursor study, biochemical analysis of the hepatopancreas, moisture content, protein- and lipid concentrations showed the required seasonality. However, of the “peak” values, only lipid concentration correlated with growth. It was therefore described as a simple and robust predictor for adult male growth in *J. lalandii*, based on measurements conducted from 1991-1995 in which the lipid content correlated with moult increment of the same year (Cockcroft,

1997). It was therefore recommended for inclusion into resource management as an indicator of the current year's growth increment but has not been implemented into stock assessment procedures more than twenty years later. Here, no such correlation was found: Lipid concentration was substantially higher in 2010 than in the following years. However, at least compared with 2011, this did not correlate with a higher growth rate. In addition to less pronounced seasonal changes and drawn-out individual life cycle events (see above), this could be a result of gaps in sampling that occurred in the present study due to logistical problems in some years. Concluding from the present results, sampling without gaps is necessary during all peak months, presumably from December to May. The course of hepatopancreas protein content (per wet weight and dry weight) is also strongly seasonal, qualifying for consideration as potential indicator. Unfortunately, in 2012, when protein content was higher during peak months, the corresponding growth value was only estimated. Protein quantitation is less labor-intensive than that of lipids and can be automated to a large extent. Its use as an indicator would therefore be of advantage. Correlation of both relative lipid and protein contents with HSI are significant but have a relatively low slope (Fig. 8B,C). It may indicate that a potential indicator could be found in the HSI, if analyzed differently. This may require determining the difference between peak- and bottom values and also requiring sampling from July to September.

Data from July of both years are the last data collected before moult. In addition, lipid concentration is highest in July of each sampling year, indicating possible usefulness as a predictor for growth increment in the subsequent moult. July data were therefore analysed in more detail. To obtain a more meaningful value, the highly seasonal hepatopancreas size was included in this analysis although July is several months after the proposed peak accumulation in March to May when HSI reaches its peak. The total amount of lipid per organ (hepatopancreas) available for growth was obtained in this way. The calculated total amount of lipids per hepatopancreas showed a clear seasonal trend, a prerequisite for suitability as an indicator (Cockcroft, 1997). However, this curve also revealed a peak in July 2011 but not in July 2010. Despite this, comparison of July data from both years revealed differences that coincide with growth increment: HSI, lipid content and lipid per hepatopancreas are all significantly higher in 2011 than in 2010, which correlates with 26% (0.7 mm) faster growth during subsequent moult in 2011 than in 2010 (Table 4). In 2010, lipid per hepatopancreas declined already before July, possibly indicating their use for other purposes and suboptimal food availability and hence resulting in a slower growth rate. In July 2011, hepatopancreas protein concentration is lower than in July 2010 (Fig. 5). As described above, it is inverse to

lipid concentration and also displays a strong seasonal trend. It may in future be used for a growth predictor, too.

3.5. Conclusion

The present study confirms seasonal accumulation trends of metabolic reserves in male *J. lalandii* from a fast growth and a slow growth area. However, previously reported potential indicators for annual male growth were not robust enough here, possibly due to different seasonal reserve accumulation. Moreover, an attempt to use moult stage to identify a growth predictor failed. Instead, various lipid data from the immediate pre-moult period (July sampling point in the present study) appeared as more promising growth predictors. More research is required to confirm this finding and to add a quantitative component. For such research, sampling for "peak accumulation" should ideally span from December to May each season whereas sampling for lipid indicators should take place from July to September.

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Chapter 4

Variation in biological and biochemical parameters in mature female spiny lobsters *Jasus lalandii* in relation to growth and reproduction

Abstract

Biological body parameters of mature female West Coast rock lobsters, *Jasus lalandii*, were analysed over two years to determine accumulation of reserves during two moult- (i.e. growth-) and ovarian (i.e. reproductive-) cycles. Two historically fast- and slow male growth fishing areas were compared, respectively. Male growth was suggested as a predictor for female fecundity and *vice versa*. In addition, the progression of biochemical composition of hepatopancreas and ovaries was determined for the fast growth area together with moult stage and ovarian maturation. This information was intended to confirm previously proposed predictors of annual male moult increment and identify potential new ones. In addition, the partitioning of metabolic reserves between growth and reproduction was analysed. The present study confirmed seasonal accumulation trends of metabolic reserves in gonads and hepatopancreas of female *J. lalandii*. Analysis on the partitioning of reserves between gonads and hepatopancreas during ovarian maturation revealed little or no reserve accumulation in hepatopancreas prior to peak ovary reserve accumulation. The study could not provide robust indicators for predicting male growth increment in the subsequent moult. However, average relative ovary size (GSI) was consistently higher in the high growth fishing area and the amount of lipids per ovary was highly seasonal. These are potential directions for future research in this regard.

Keywords: Spiny lobster, palinurid, physiological growth predictor, reserve accumulation, ovary maturation, moult

4.1 Introduction

The fishery of the West Coast rock lobster (WCRL), *Jasus lalandii,* is one of the most important in South Africa due to its high commercial value and its high employment provision for impoverished communities. The Branch: Fisheries of the Department of Forestry, Fisheries and the Environment, (DFFE) manages the heavily depleted WCRL resource according to an operational management procedure (OMP) since 1997 (DEFF, 2020). This includes a Total Allowable Catch (TAC) and additional effort control. Most of these measures are based on male lobsters, which form the bulk of the resource that is legally harvested. A closed season regulation is in place in this fishery to protect females in the months when they are in berry (i.e., carrying eggs under their abdomen).

Mature WCRL moult and reproduce once a year and the life cycles of males and females are highly synchronized (Pollock, 1986). Females moult in late austral autumn to early winter (April-June) and mate shortly thereafter (July-August) (Heydorn, 1969), when the female exoskeleton is still relatively soft and pleopodal setae are fresh for egg attachment (Jeffs et al., 2013; Munian et al., 2021). In contrast to males, which use 99% of energy reserves accumulated during intermoult for growth, females use 95% for reproduction (Zoutendyk, 1990). Available energy metabolites in females, derived from food intake and stored reserves, need therefore be partitioned between reproduction and growth. The two most important female storage organs for these processes are the hepatopancreas (as in males) and the female gonads (ovaries). Energy reserves in males and females are stored in the hepatopancreas (or midgut gland), mainly as lipids and proteins (Cockcroft, 1997; van Rooy, 1998). During the build-up of reserves, the maturing ovaries of female crustaceans become a second centre of energy accumulation (Harrison, 1990). In males, energy accumulation in the hepatopancreas takes place between the annual moulting events (Cockcroft, 1997). The situation in females is more complex since reproduction is a precisely regulated process under endocrine control (Jayasankar et al., 2020). Lipids accumulate together with proteins in the maturing and growing ovaries, indicated by increasing size until spawning (Harrison, 1990; van Rooy, 1998). In the hepatopancreas, however, there is no similarly clear trend through the moult cycle as in males (van Rooy, 1998).

The synchronization of male and female life cycles in *J. lalandii* means that the metabolic reserves in the male hepatopancreas and female gonads build up during the same time period (Cockcroft, 1997), as supported by the positive relationship between male growth and female

fecundity found by Melville-Smith et al. (1995). Environmental influences therefore influence both processes simultaneously (Cockcroft, 1997). Fecundity of female *J. lalandii* varies along the Cape West Coast with greater fecundity (more eggs) produced in areas where somatic growth is fast (Beyers and Goosen, 1987). In times of nutritional stress, *J. lalandii* females sacrifice growth to optimize egg production (Cockcroft, 1997). A reduced lipid accumulation during intermoult by males of this species was shown to lead to low growth rates and even shrinkage (Cockcroft and Goosen, 1995; Cockcroft, 1997), possibly the reason for the observed sharp decline in productivity of the resource towards the end of the 1980s (Shannon et al., 1992; Pollock et al., 1997). A similar impact on reserve accumulation and maturation in ovaries most likely contributed to this phenomenon.

Dynamics of lipids and proteins in decapod ovaries and hepatopancreas are interlinked. Their accumulation in ovaries is a result of vitellogenesis (production of vitellogenin and lipoprotein and their storage by oocytes) in the ovaries themselves but possibly also with contribution from the hepatopancreas from where they are subsequently transferred to the ovaries via the haemolymph (Harrison, 1990). In palinurids, however, there is indirect evidence for the closely related *J. edwardsii* that lipid demand of ovarian maturation is largely met through dietary intake and not only (or not at all) from the hepatopancreas (Smith et al., 2004). Similar conclusions were found for several crab species (Hasek and Felder, 2005, 2006). If this is true for *J. lalandii*, food shortage during ovary maturation has a direct impact on reproductive success. In particular, the impact during the reserve accumulation phase is important, because starvation or sub-optimal food supply may affect biological events (moult, spawning) long before they happen.

For the present study, various biological parameters of females were used to monitor and analyze progress in the moult- and reproductive cycles: During the moult cycle, metabolites for growth are accumulated in the hepatopancreas of male *J. lalandii* and the relative hepatopancreas size (Hepatosomatic Index = HSI) is therefore a good indicator of this process (see previous chapter). Similarly, vitellogenesis results in a large increase in relative ovary size (Gonadosomatic Index = GSI) and therefore describes the accumulation of metabolic reserves during maturation of female gonads for reproduction (van Rooy, 1998). In addition, ovaries can easily be staged by determination of shape, surface structure and colour (Heydorn, 1969). Determination of new exoskeleton formation by setagenic analysis of pleopods (Marco, 2012) is a relatively simple but accurate method to determine the various stages of the moult cycle.

Currently, little or no information is available on biochemical changes during moult- and reproductive cycles in female *J. lalandii* (and palinurids in general).

This chapter aimed at determining and comparing the seasonal variation in the moult- and ovary cycles of a high- (Hout Bay) and a low- (Olifantsbos) growth rates of WCRL fishing areas. In addition, biochemical analysis of hepatopancreas and ovaries from the fast-growing area targeted details of reserve accumulation- and partitioning between growth (hepatopancreas) and reproduction (ovaries). The data were also examined to determine their potential as predictors of male somatic growth.

4.2 Materials and methods

Female rock lobsters (n = 7 - 30, but for most months n =25-30) of 57 - 95 mm carapace length (CL) was caught more or less monthly, depending on availability of ship's time and sea conditions, at two sites on the South African west coast (see Chapter 3) from January 2010 to March 2012. Only sexually mature female lobsters were used; size at which differs slightly by area (Cockcroft and Goosen, 1995). Even though all sexually mature females were kept, in some months it was difficult to get sufficient numbers. There was therefore no preselection for size. The Hout Bay site ("Die Knol"; 34º04'S, 18º20'E), located in a rock lobster sanctuary, is historically a fast lobster growth rate area, whereas the Olifantsbos site (34°16'S, 18°22'E), in a commercially exploited area, is characterized by slow lobster growth rates (Cockcroft, 1997). Lobsters were sampled from the research vessel RV "Ellen Khuzwayo" with the aid of standard commercial gear (Pollock, 1986) at depths of approximately 80 to 200 m. After capture, Total wet weight (w_T) and CL were measured. w_T was determined by digital balance, whereas Vernier callipers were used to measure CL (from rostrum to midpoint of posterior edge of carapace). Animals were subsequently sacrificed and dissected. Abdominal (tail) weight (w_A) was determined after dissection, whereas the hepatopancreas and gonads were transferred into pre-weighed vessels. Samples were then blast-frozen aboard immediately and later transported to freezers at the laboratory where they were kept at -40 °C for later biochemical analysis and determination of weight. They remained sealed from air in a plastic vial to prevent auto-oxidation of the lipids in the sample. For determination of moult stage, a pleopod was sampled from some batches of lobsters, which were individually placed in an Eppendorf tube for storage at -25 °C.

Biochemical analyses were performed by the Council of Scientific and Industrial Research (CSIR) of South Africa as follows: After removal from the freezer, hepatopancreas weight (w_H)

and gonad weight (w_O) were determined, the sample defrosted and homogenized using a Ultraturrax blender (IKA, Germany). Subsequently, moisture content (by heating for 24 hours at 104 °C), crude protein (LECO protein analyzer, measurement of nitrogen by standard DUMAS; % nitrogen was converted to % protein using the factor 6.25) and total lipid content (Bligh and Dyer 1959) were determined from aliquots. Ash and carbohydrate contents were determined by difference (% Carbohydrates and Ash = 100 % - sum of (% lipids + % moisture + % protein).

Somatic indices, i.e., hepatosomatic index (HSI), tail index (TI) and gonadosomatic index (GSI) were calculated according to equations:

$$I. \quad HSI = \frac{Hepatopancreas\ wet\ weight}{Total\ body\ weight} \times 100\%$$

$$II. \quad TI = \frac{Tail\ wet\ weight}{Total\ body\ weight} \times 100\%$$

$$III. \quad GSI = \frac{Gonad\ wet\ weight}{Total\ body\ weight} \times 100\%$$

The stage of individual lobsters in the moult cycle was determined via microscopic analysis of setagenic features of pleopods as outlined in Marco (2012) for adult *J. lalandii*. Briefly, the distal section of the pleopods were floated on a cavity slide, covered with a cover slide, and examined with a compound eclipse Ni microscope (Nikon, Japan). The moult stages of *Jasus lalandii* are divided into stages AB (postmoult), C (intermoult), D_0 - $D_{3\text{-}4}$ (premoult)(Marco, 2012). Substages of premoult stage D_1, such as D_1', D_1", were lumped together for simplicity and all moult stages were later coded as numerical values for analytical purposes (C = 1, D_0 = 2, D_1 = 2.5, D_2 = 3). Ovaries were staged by maturity according to Heydorn (1969). In brief, ovaries were removed and classified from stages 1 (immature) to 6 (spent) according to coloration and size as detailed in Table 1. The numerical value was used for further statistical analysis, for example for the relative contribution of each ovarian stage to the total.

Statistical differences of biological and biochemical parameters between ovary- and moult stages, respectively, were analyzed by one-way ANOVA followed by the appropriate parametric- or nonparametric post-hoc test (given in figure captions), depending on normality of sample distribution. Linear regression statistics were used for regression analysis of organ biochemistry. Statistics were determined using Sigma Plot version 14.0. The significance level chosen throughout was $p < 0.05$.

Table 1. Macroscopic classification of *J. lalandii* ovarian maturity according to Heydorn (1969).

Stage of maturity	Number code	Colour	Weight	Macroscopic appearance of ovary
Immature	1	White	0.1 – 1.0 g	No discrete ova visible
Inactive to Active	2	Cream → yellow → peach	1.0 – 3.9 g	Starts to swell, no discrete ova visible
Active	3	Orange → light brown	4.0 – 8.0 g	More swollen and convoluted, fills ~2/3 of cephalothoracic cavity, discrete ova slightly visible
Active to Ripe	4	Brown → deep red	5.0 – 12.0 g	More convoluted. extends full length of cephalothoracic cavity, discrete ova visible
Ripe	5	Dark brick-red	7.0 – 15.0 g	More convoluted than in the previous stage, posterior horns reach first abdominal segment, discrete ova clearly visible
Spent	6	White → grey	0.6 – 2.0 g	Few residual ova may be identified as red spots, size decreased, and convolutions lost

4.3 Results

Biological data

The samples from the two sites show clear differences in size and weight range (Table 2). Female lobsters from the Hout Bay ranged from 176 – 236 g in mean total weight (CL 67 – 75 mm) whereas in Olifantsbos, total weight averaged 145 – 198 g (CL 63 – 70 mm). Similarly, mean wet tail (abdomen) weight w_A ranged from 63 – 93 g (with average TI from 33 – 41%) in Hout Bay and from 53 – 83 g (average TI 35-42%) in Olifantsbos. In both areas the average TI values showed no clear seasonal pattern although lowest values in both areas were noted towards the end of each calendar year (Table 2, Fig. 1A and 2A.

The mean w_A/CL ratios in Hout Bay (0.9 to 1.3) and Olifantsbos (08. -1.2) were similar throughout the sampling period (Table 2, Fig. 1B and 2B). For both areas, values were lowest in austral autumn and highest in winter/spring.

In both areas, the mean hepatopancreas wet weight (w_h) was heaviest over the summer months and lightest in the remaining seasons (Table 2). There was no clear trend in the HSI from both areas with values in Hout Bay ranging from 4 -5.1% and from 3.6 – 5.2% in Olifantsbos (Table 2, Fig. 1C and 2C).

Table 2. Biological data of female *J. lalandi* caught at two sites from March 2010 to March 2012.

Date	n		Total wet weight	Tail wet weight	Tail Index	Hepatopancreas wet weight	Hepatosomatic Index	Gonad wet weight	Gonadosomatic Index	Tail weigt : Carapace Length	Moult Stage	Gonad stage
		CL	w_T	w_A	TI		HIS		GSI	w_A/CL	numerical	numerical
		mm	g	g	% of w_T	G	% of w_T	g	% of w_T			
Hout Bay												
Mar 2010	16	68.7 ± 3.2	189.1 ± 24.8	70.9 ± 10.8	37.4 ± 1.7	8.0 ± 1.3	4.2 ± 0.3	7.7 ± 2.7	4.0 ± 1.2	1.0 ± 0.1	1.7 ± 0.5	3.2 ± 0.4
Apr 2010	30	68.9 ± 4.5	196.8 ± 37.0	74.9 ± 16.3	38.0 ± 2.1	7.8 ± 1.6	4.0 ± 0.6	8.6 ± 2.9	4.5 ± 1.4	1.1 ± 0.2	n.d.	3.4 ± 0.8
May 2010	30	72.6 ± 3.6	224.2 ± 26.0	93.2 ± 12.4	41.6 ± 2.7	10.5 ± 2.4	4.6 ± 0.8	3.5 ± 3.7	1.6 ± 1.9	1.3 ± 0.1	n.d.	2.5 ± 1.1
Jul 2010	30	71.0 ± 3.7	224.1 ±30.0	88.9 ± 13.5	39.6 ± 2.2	8.9 ± 1.9	4.0 ± 0.5	2.5 ± 2.3	1.1 ± 1.0	1.2 ± 0.1	1.4 ± 0.5	2.3 ± 0.8
Oct 2010	24	71.3 ± 4.2	220.7 ± 34.0	88.3 ± 13.2	40.0 ± 3.8	9.0 ± 2.0	4.0 ± 0.7	3.5 ± 2.2	1.5 ± 1.0	1.2 ± 0.1	1.9 ± 0.5	2.6 ± 0.6
Nov 2010	25	70.5 ± 4.1	216.3 ± 34.2	81.6 ± 14.1	37.7 ± 2.3	10.7 ± 1.9	5.0 ± 0.7	3.9 ± 1.7	1.8 ± 0.7	1.2 ± 0.2	1.8 ± 0.6	3.1 ± 0.5
Dec 2010	25	73.2 ± 3.2	233.4 ± 28.2	85.0 ± 10.9	36.4 ± 1.6	11.9 ± 1.7	5.1 ± 0.4	6.8 ± 2.2	2.9 ± 0.7	1.2 ± 0.1	1.7 ± 0.5	3.4 ± 0.5
Jan 2011	25	70.4 ± 3.1	205.9 ± 26.2	77.0 ± 10.3	37.4 ± 2.1	10.2 ± 1.6	4.9 ± 0.5	7.1 ± 2.3	3.4 ± 0.8	1.1 ± 0.1	1.7 ± 0.6	3.1 ± 0.3
Feb 2011	25	71.0 ± 2.3	209.5 ± 23.4	79.0 ± 11.6	37.6 ± 3.2	9.1 ± 1.1	4.4 ± 0.5	8.2 ± 2.3	3.9 ± 1.0	1.1 ± 0.1	1.8 ± 0.5	3.3 ± 0.6
Apr 2011	7	67.0 ± 1.4	176.3 ± 16.6	63.4 ± 9.1	35.9 ± 2.7	8.5 ± 0.6	4.8 ± 0.5	6.0 ± 0.9	3.4 ± 0.4	0.9 ± 0.1	1.9 ± 0.6	3.4 ± 0.5
May 2011	20	70.4 ± 2.7	201.2 ± 20.3	75.5 ± 9.1	37.5 ± 2.4	9.2 ± 2.1	4.6 ± 0.8	7.6 ± 3.9	3.8 ± 2.0	1.1 ± 0.1	n.d.	3.6 ± 1.0
Jul 2011	25	69.6 ± 3.2	211.4 ± 24.7	87.0 ± 12.3	41.1 ± 2.6	9.5 ± 1.8	4.5 ± 0.8	1.8 ± 0.5	0.8 ± 0.2	1.2 ± 0.1	1.3 ± 0.5	2.3 ± 0.5
Oct 2011	25	72.0 ± 4.5	225.0 ± 32.2	92.8 ± 11.8	41.4 ± 2.4	9.9 ± 1.3	4.5 ± 0.7	4.8 ± 1.0	2.2 ± 0.5	1.3 ± 0.1	1.7 ± 0.6	2.2 ± 0.4
Nov 2011	25	74.7 ± 5.0	234.8 ± 48.3	77.6 ± 16.1	33.1 ± 0.0	10.5 ± 2.3	4.5 ± 0.7	5.4 ± 1.9	2.3 ± 0.5	1.0 ± 0.1	1.8 ± 0.6	2.9 ± 0.4
Dec 2011	22	74.7 ± 6.3	236.0 ± 54.0	85.7 ± 18.4	36.5 ± 2.3	10.1 ± 2.0	4.3 ± 0.5	7.4 ± 2.2	3.1 ± 0.5	1.1 ± 0.1	2.3 ± 0.3	3.1 ± 0.5
Jan 2012	25	71.6 ± 4.7	207.9 ± 25.7	78.9 ± 9.0	38.0 ± 1.5	9.8 ± 2.0	4.7 ± 0.7	6.8 ± 2.5	3.3 ± 1.3	1.1 ± 0.1	2.3 ± 0.4	3.1 ± 0.4
Feb 2012	25	70.2 ± 4.4	203.5 ± 31.1	76.0 ± 11.3	37.4 ± 2.3	9.1 ± 2.1	4.4 ± 0.7	8.2 ± 2.4	4.0 ± 0.8	1.1 ± 0.1	2.2 ± 0.6	3.6 ± 0.6
Mar 2012	25	71.1 ± 3.0	207.9 ± 25.7	78.9 9.0	38.0 ± 1.5	9.8 ± 2.0	4.7 ± 0.7	8.6 ± 2.4	4.2 ± 1.2	1.1 ± 0.1	2.0 ± 0.4	3.8 ± 0.5
Olifantsbos												
Mar 2010	30	66.5 ± 4.0	178.4 ± 31.4	66.2 ± 12.6	37.1 ± 1.6	7.5 ± 1.7	4.2 ± 0.6	6.7 ± 2.7	3.7 ± 1.3	1.0 ± 0.1	n.d.	4.1 ± 0.5
Apr 2010	30	68.5 ± 4.0	190.0 ± 29.0	71.0 ± 11.0	37.4 ± 1.7	8.6 ± 1.8	4.5 ± 0.7	8.0 ± 3.0	4.2 ± 1.3	1.0 ± 0.1	n.d.	3.6 ± 0.5
May 2010	30	67.2 ± 4.4	182.2 ± 37.3	69.0 ± 17.0	37.7 ± 2.3	7.9 ± 1.7	4.4 ± 0.6	7.5 ± 3.2	4.3 ± 1.7	1.0 ± 0.2	n.d.	3.7 ± 0.7
Jul 2010	30	69.8 ± 3.6	198.5± 45.5	80.2 ± 12.7	39.1 ± 1.8	7.5 ± 2.1	3.6 ± 0.6	2.7 ± 3.0	1.4 ± 1.6	1.1 ± 0.1	n.d.	2.3 ± 0.8
Oct 2010	30	68.2 ± 3.1	180.5 ± 23.3	67.1 ± 8.9	37.2 ± 1.5	8.0 ± 1.8	4.4 ± 0.6	2.9 ± 1.3	1.6 ± 0.6	1.0 ± 0.1	n.d.	3.0 ± 0.7
Nov 2010	30	68.3 ± 2.7	189.0 ± 22.6	71.2 ± 8.1	37.6 ± 1.3	9.6 ± 1.7	5.1 ± 0.5	3.4 ± 1.3	1.8 ± 0.8	1.0 ± 0.1	n.d.	3.0 ± 0.5
Dec 2010	30	64.9 ± 2.2	161.3 ± 13.7	58.6 ± 4.8	36.5 ± 1.8	7.7 ± 1.5	4.8 ± 0.8	1.9 ± 0.8	1.3 ± 0.6	0.9 ± 0.1	n.d.	2.9 ± 0.7
Jan 2011	30	64.9 ± 2.5	163.3 ± 17.0	60.0 ± 6.9	36.2 ± 2.0	8.0 ± 1.3	5.0 ± 0.7	2.6 ± 1.1	1.6 ± 0.6	0.9 ± 0.1	n.d.	3.0 ± 0.6
Feb 2011	30	68.0 ± 2.8	182.9 ± 16.5	67.1 ± 5.5	36.9 ± 1.5	9.0 ± 1.3	4.8 ± 0.6	5.7 ± 2.0	3.2 ± 1.1	1.0 ± 0.1	n.d.	3.6 ± 0.6
Mar 2011	30	67.7 ± 2.6	176.6 ± 20.2	62.4 ± 7.0	35.4 ± 1.4	8.4 ± 1.3	4.8 ± 0.7	4.4 ± 1.5	2.5 ± 0.9	0.9 ± 0.1	n.d.	3.6 ± 0.6
Apr 2011	30	64.3 ± 2.4	156.1 ± 14.3	57.0 ± 6.3	36.5 ± 1.8	8.1 ± 1.4	5.2 ± 0.6	4.6 ± 1.2	4.6 ± 1.2	0.9 ± 0.1	n.d.	3.2 ± 0.8
May 2011	20	63.4 ± 2.5	145.4 ± 15.8	52.5 ± 7.3	36.1 ± 2.7	7.1 ± 1.1	4.9 ± 0.7	4.8 ± 0.8	3.3 ± 0.6	0.8 ± 0.1	n.d.	3.8 ± 0.6
Jul 2011	30	67.8 ± 2.5	181.4 ± 15.9	68.2 ± 5.3	37.3 ± 2.1	7.7 ± 1.2	4.3 ± 0.6	9.3 ± 2.7	5.1 ± 1.3	1.0 ± 0.1	n.d.	3.4 ± 0.5
Oct 2011	24	68.0 ± 4.6	197.4 ± 32.2	82.6 ± 13.4	42.0 ± 3.4	8.3 ± 1.7	4.2 ± 0.5	1.8 ± 1.2	0.9 ± 0.5	1.2 ± 0.2	n.d.	2.3 ± 0.4
Nov 2011	30	68.1 ± 3.1	197.2 ± 21.5	75.6 ± 5.8	38.6 ± 3.5	8.7 ± 1.7	4.4 ± 0.6	4.2 ± 1.2	2.1 ± 0.5	1.1 ± 0.1	n.d.	3.0 ± 0.6
Dec 2011	30	66.5 ± 3.2	186.4 ± 12.1	69.0 ± 6.3	37.0 ± 2.3	8.9 ± 1.5	4.7 ± 0.6	4.8 ± 1.3	2.5 ± 0.7	1.0 ± 0.1	n.d.	3.1 ± 0.4
Jan 2012	30	67.2 ± 2.7	179.0 ± 11.6	65.1 ± 5.6	36.4 ± 2.3	7.9 ± 1.6	4.4 ± 0.7	5.4 ± 1.4	3.0 ± 0.8	1.0 ± 0.1	n.d.	3.5 ± 0.6
Feb 2012	30	66.8 ± 3.5	169.6 ± 27.1	63.5 ± 8.9	37.7 ± 4.3	7.0 ± 1.3	4.2 ± 0.8	6.5 ± 2.1	3.8 ± 1.1	1.0 ± 0.1	n.d.	3.8 ± 0.4
Mar 2012	30	64.0 ± 3.2	149.4 ± 16.4	56.9 ± 7.1	38.0 ± 2.2	7.4 ± 1.3	4.9 ± 0.5	3.6 ± 0.8	2.4 ± 0.6	0.9 ± 0.1	n.d.	3.3 ± 0.5

Values are given as mean ± SD. n.d. = not determined. Abbreviations: w_T = total wet weight, w_A = tail wet weight, w_O = ovary wet weight, w_H = hepatopancreas wet weight, TI = tail index, HSI = Hepatosomatic Index, GSI = Gonadosomatic index.

Moult cycle

The stage in the moult cycle was determined from some batches (months) of female lobsters in the Hout Bay area. Analysed animals were mainly in intermoult stage C and premoult stages D_0 and D_1; very few were in premoult stage D_2 (Fig. 3A). Stage D_1 consists of three sub-stages; for simplicity and given the relatively small sample size, D_1 was not further divided, and all sub-stages are summarized as D_1. Lobsters in stage C were present in all months sampled except December 2011. Numbers ranged from 1 to 19 with the highest number in July 2010 (63%) while the lowest was found in January and March 2012 (4% in each month). The highest percentage of lobsters in stage C was in July 2011 (72%). The number of lobsters in stage D_0 ranged from 1 to 18 (6 - 72%) and was present throughout each months' samples. The share of the stage was low in July 2010 (33%), July 2011 (28%) and March 2012 (28%). In all other months, it reached 43 – 72%. The number of lobsters in stage D_1 ranged from 1 to 16 (3 - 64%) and was the dominant stage in December (55%) and January 2011 (52%) and March 2012 (64%). D_1 was not observed in 4 of the 15 months sampled. The highest share was recorded in July 2010 (37%) and October 2011 (28%). Lobsters in stage D_2 were only found in 4 of the sampled months; their number was very small, ranging from 1 - 3 (4 - 12%) and occurred over year-end of both sampling seasons.

To achieve a clearer trend by possibly reducing yearly variation, moult stage numbers were accumulated, where possible, for the same calendar months of the whole sampling period (Fig. 3B). These accumulated results show that lobsters caught throughout the year are mainly in stages C and D_0 (59 – 98%) and more advanced premoult stages D_1 and D_2 occurred mostly in January (32%) to April (27%) with the highest observed in March (32%). Lowest occurrence was in July (2%) after which it increased again to 25% in December. To present the above data in a simple, continuous trend, moult stages were coded and converted to numerical values (Table 2, Fig. 3C). Early in the moult cycle (stage C), values would be low and increase as the cycle progresses. The lowest values were recorded in July 2010 (1.4) and July 2011 (1.3), whereas the highest were found from December 2011 (2.3) to March 2012 (2.3).

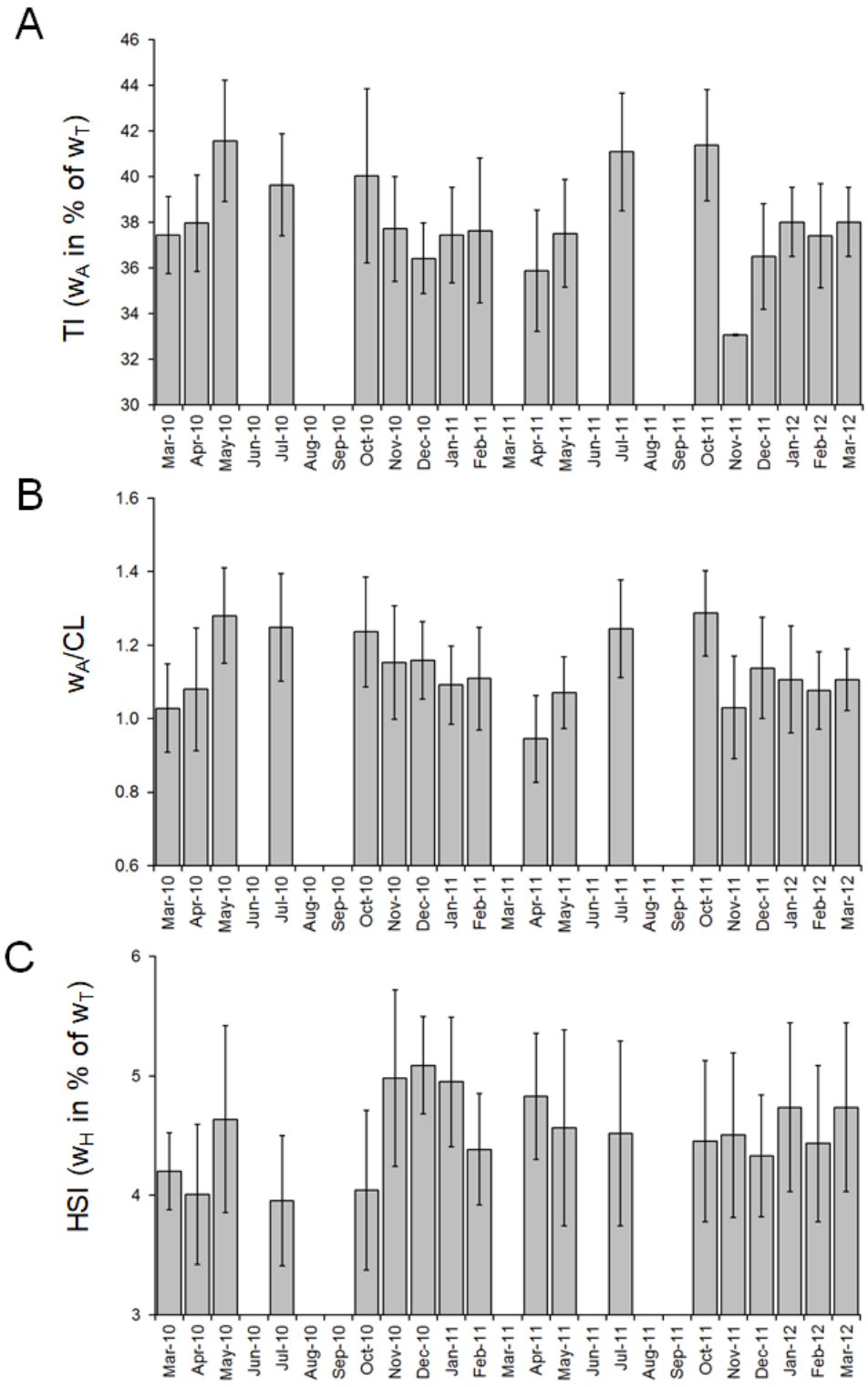

Figure 1. The course of biological parameters from female *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. A) Tail Index (TI), B) ratio of tail weight and Carapace length (w_A/CL) and C) Hepatosomatic Index (HSI). Values are means ± S.D., n = 7-30. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

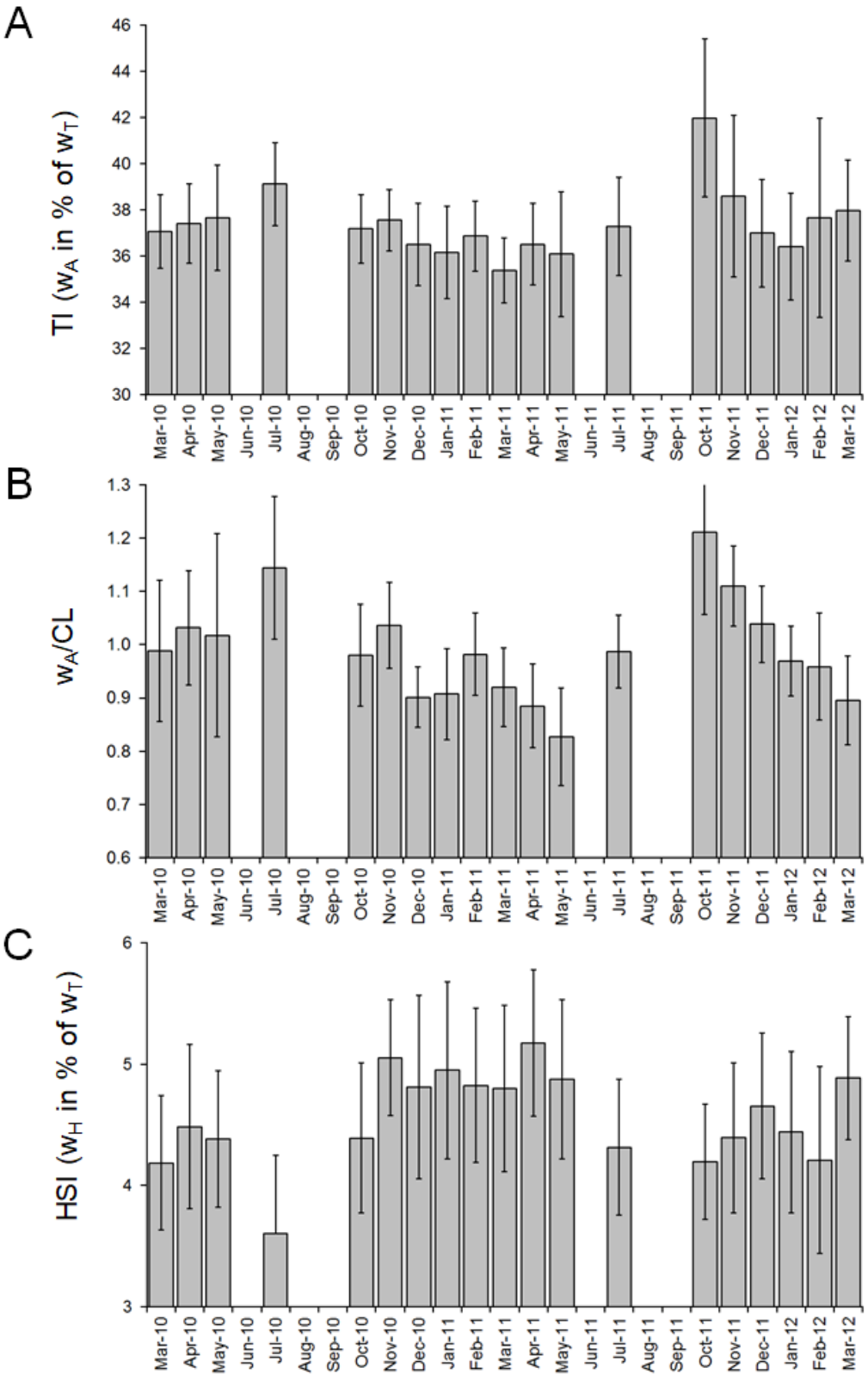

Figure 2. The course of biological parameters from female *J. lalandii* caught at the Olifantsbos sampling site during the period from March 2010 to March 2012. A) Tail Index (TI), B) ratio of tail weight and Carapace length (w_A/CL) and C) Hepatosomatic Index (HSI). Values are means ± S.D., n = 24 - 30. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

Ovarian cycle

In both areas, the mean ovary wet weight (w_g) was heaviest over the summer months and lightest in the remaining seasons (Table 2). In Hout Bay w_g fluctuated between 1.8 and 8.6 g and between 1.9 – 9g in Olifantsbos. Although the weight ranges in the two areas were similar in general, values in Olifantsbos were lighter than in Hout Bay, most probably due to the smaller size of the lobsters caught in Olifantsbos (Table 2).

The mean Gonadosomatic Index (GSI) in Hout Bay ranged from 0.8 – 4.5 % and from 1.4 – 5.1% in Olifantsbos. (Table 2, Fig. 4A and 5A). The frequency of each gonad stage during the seasonal ovarian cycle is depicted in Figs. 4C and 5C for each area (as % of all analyzed ovaries). In both areas the frequency of ovaries in the later stages of maturity (stages 4 and 5) were highest in late summer and autumn and lowest in winter. The frequency of the earlier stages of ovarian maturity (stages 1 and 2) were conversely highest in winter and lowest in summer. This is also reflected in the composite gonad stage value (and GSI indices) for each area (Table 2, Figs. 4 AB, 5AB). While the general trend of ovary maturation was similar in both areas, the high frequency of later stage ovaries recorded in the Olifantsbos area in July 2011 indicated a later spawning than in 2010. No lobsters with gonads at stage 6 (spent) were found in either sampling area.

Ovary biochemistry

Moisture content: The mean moisture content of the gonads is inverse to w_d. Dry weight is therefore not reported here but serves as reference value for the content of other compounds (Table 3). Moisture content ranged from 46.4 – 65.4% with highest values recorded in austral winter each year, after which they declined until late summer/autumn (Table 3; Fig. 6A).

Lipid content: Lipid content ranged between 22.3 – 49.3% with lowest values recorded during winter and then increasing to intermediate levels during summer and peaking in early autumn. (Tab. 3; Fig. 6B).

Protein content: Protein content follows a similar trend to lipid content, albeit with less fluctuations (Fig. 6B). Lowest protein values of approximately 25 - 33% were recorded in July/October 2010 and July 2011. Highest values of more than 45 - 48% occurred during summer of both seasons.

Carbohydrate and ash content: There was no clear trend in the seasonal course of these compounds, although in each July the highest value of 45 – 50 % was recorded. Lowest values of about 5% occurred in autumn 2011. Individual fluctuations of values were large (Table 3).

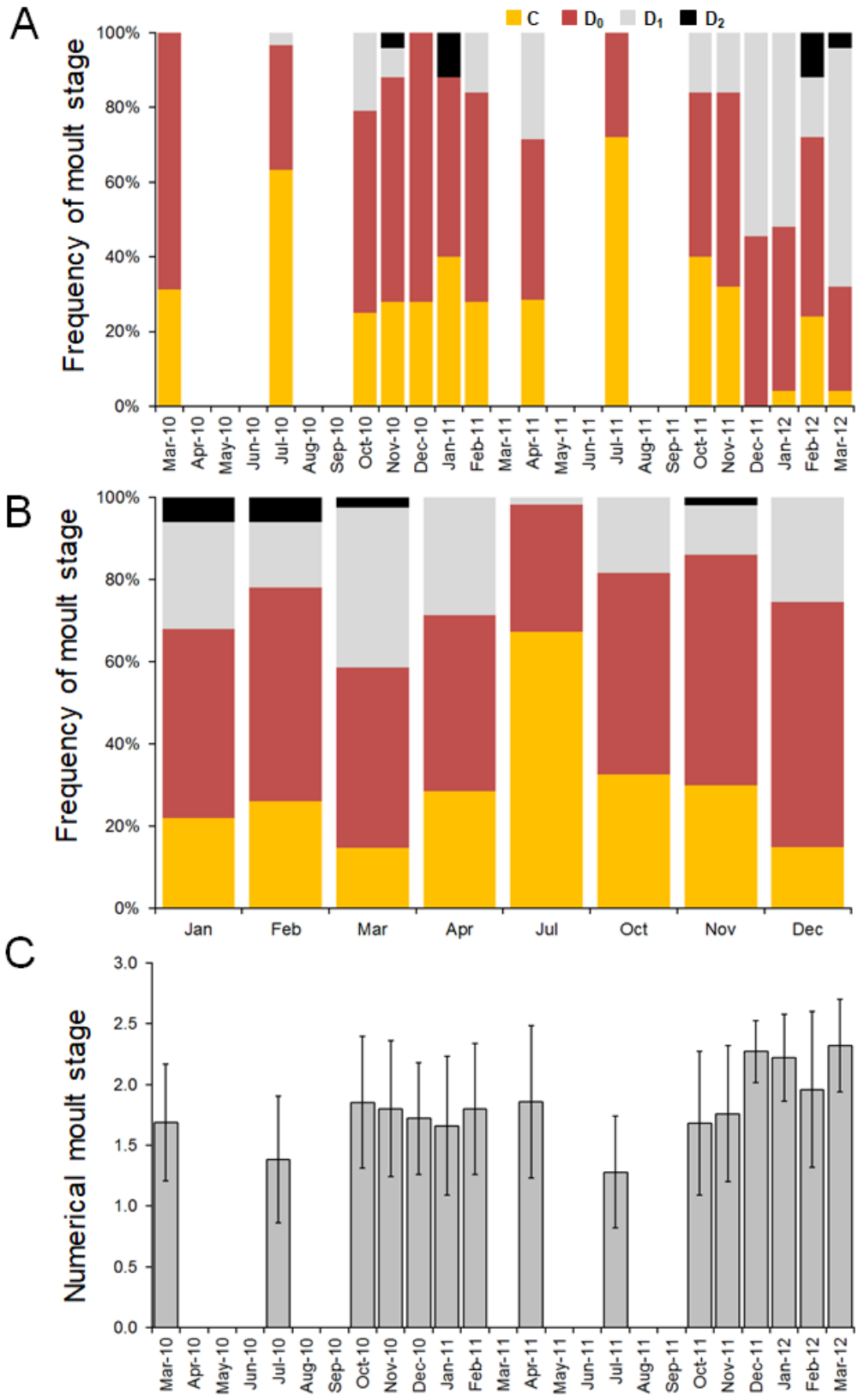

Figure 3. Moult stage of female *J. lalandii* caught at the Hout Bay sampling site ("Knol") during select months during March 2010 to March 2012. A) Numerical course of stage in moult cycle throughout the months of the sampling period, B) monthly frequency of moult stages and C) accumulated moult stages for same months of all sampled years. In C, values are means ± S.D., n = 7 - 30.

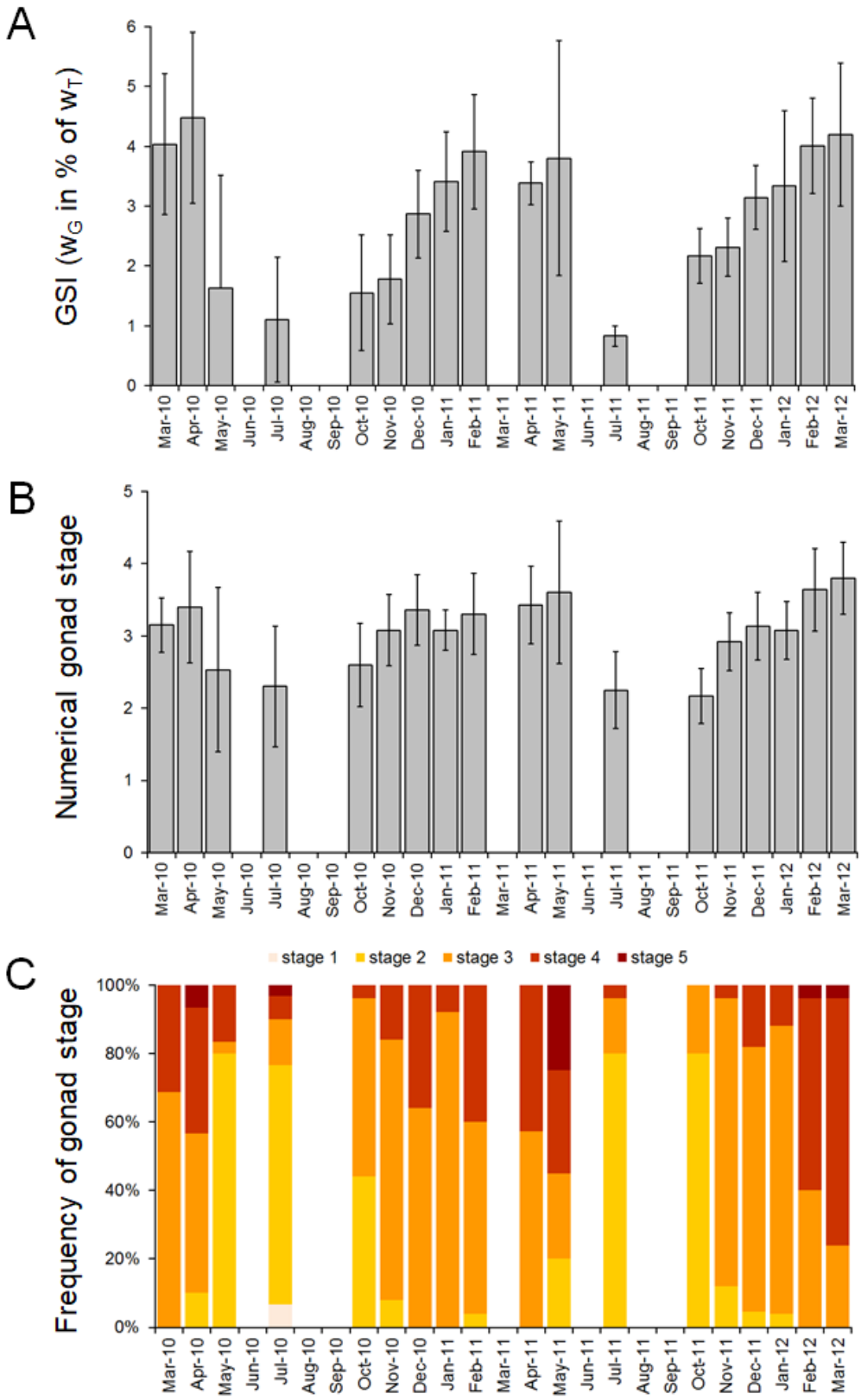

Figure 4. Seasonal course of gonad maturation from female *J. lalandii* caught at the Hout Bay sampling site and expressed as: A) relative gonad weight (Gonadosomatic Index = GSI), B) gonad stage (coded according to Table 1), C) monthly frequency of each gonad stage. In A and B, values are means ± S.D., n = 7 - 30.

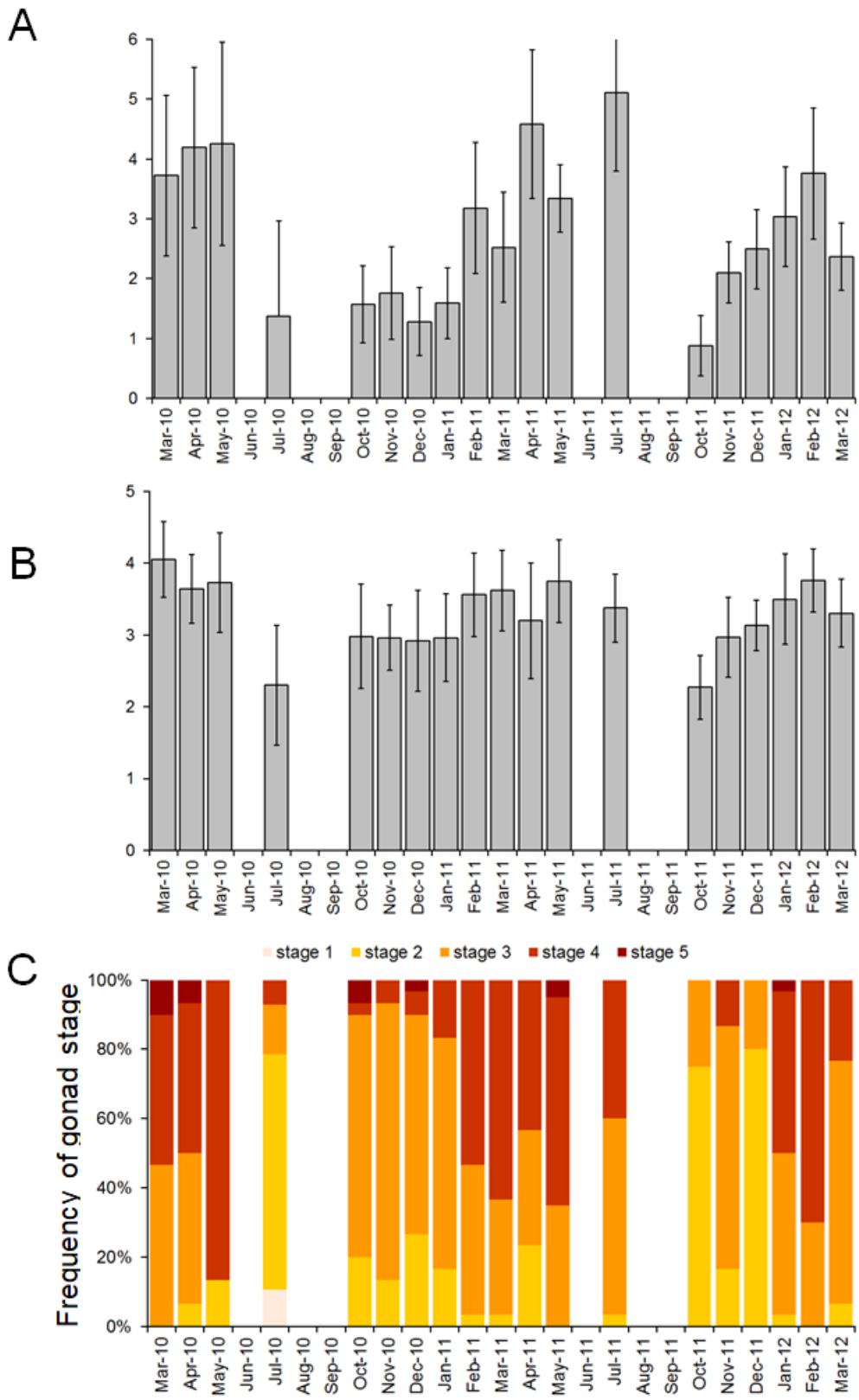

Figure 5. Seasonal course of gonad maturation from female *J. lalandii* caught at the Olifantsbos sampling site and expressed as: A) relative gonad weight (Gonadosomatic Index = GSI), B) gonad stage, C) monthly frequency of each gonad stage. In A and B, values are means ± S.D., n = 24-30.

Table 3. Biochemical composition of hepatopancreas and ovaries from female *J. lalandi* caught at the Hout Bay ("Die Knol") sampling site from March 2010 to March 2012.

Date		Moisture	w_d	Total Lipids	Proteins	Carbohydrates and ash
	n	% of w_W	% of w_W	% of w_D	% of w_D	% of w_D
Hepatopancreas						
Mar 2010	15	65.6 ± 5.8	34.4 ± 5.8	43.9 ± 6.5	42.4 ± 7.3	13.7 ± 6.9
Apr 2010	30	63.5 ± 4.2	36.5 ± 4.2	43.6 ± 9.3	39.2 ± 4.5	17.2 ± 9.6
May 2010	30	71.0 ± 3.8	29.0 ± 3.8	49.4 ± 6.7	41.5 ± 4.3	9.1 ± 5.2
Jul 2010	30	70.6 ± 3.7	29.4 ± 3.7	44.5 ± 8.0	46.5 ± 5.7	9.0 ± 7.5
Oct 2010	25	72.4 ± 3.8	27.6 ± 3.8	36.5 ± 9.9	51.0 ± 8.2	12.6 ± 9.6
Nov 2010	25	69.5 ± 3.4	30.5 ± 3.4	35.2 ± 12.2	49.0 ± 8.8	15.8 ± 6.1
Dec 2010	25	60.9 ± 1.3	39.1 ± 1.3	37.7 ± 11.8	41.3 ± 5.9	20.9 ± 9.1
Jan 2011	25	62.7 ± 5.2	37.3 ± 5.2	33.3 ± 12.5	37.9 ± 9.5	28.8 ± 10.1
Feb 2011	25	66.0 ± 4.1	34.0 ± 4.1	35.1 ± 9.6	45.9 ± 6.0	19.0 ± 11.5
Apr 2011	7	65.0 ± 2.4	35.0 ± 2.4	40.0 ± 3.6	39.5 ± 4.3	20.5 ± 7.6
May 2011	20	63.3 ± 6.0	36.7 ± 6.0	48.3 ± 13.7	38.8 ± 9.5	13.0 ± 7.9
Jul 2011	25	67.3 ± 7.2	32.7 ± 7.2	49.3 ± 12.5	34.8 ± 10.9	16.8 ± 11.5
Oct 2011	15	65.7 ± 2.3	34.3 ± 2.3	36.6 ± 4.3	28.5 ± 3.8	34.9 ± 6.4
Nov 2011	25	64.0 ± 5.3	36.0 ± 5.3	27.0 ± 10.0	54.7 ± 13.8	18.3 ± 9.0
Dec 2011	22	61.3 ± 5.1	38.7 ± 5.1	31.6 ± 18.0	44.5 ± 11.9	25.2 ± 13.0
Jan 2012	25	65.2 ± 4.8	34.8 ± 4.8	27.5 ± 17.1	55.9 ± 11.5	18.3 ± 10.6
Feb 2012	25	67.6 ± 2.9	32.4 ± 2.9	21.7 ± 7.2	46.3 ± 3.9	32.0 ± 6.9
Mar 2012	25	67.9 ± 3.4	32.1 ± 3.4	41.0 ± 9.8	45.4 ± 4.4	13.6 ± 10.5
Gonads						
Mar 2010	16	46.4 ± 8.1	53.6 ± 8.1	45.8 ± 7.7	42.9 ± 9.5	11.3 ± 7.3
Apr 2010	27	53.5 ± 7.0	46.5 ± 7.0	31.3 ± 12.3	47.5 ± 21.8	21.1 ± 10.2
May 2010	15	52.9 ± 2.9	47.1 ± 2.9	31.9 ± 3.2	37.9 ± 4.5	30.3 ± 4.3
Jul 2010	15	65.5 ± 5.7	34.5 ± 5.7	25.4 ± 7.1	25.0 ± 6.8	49.6 ± 9.4
Oct 2010	24	57.6 ± 12.9	42.4 ± 12.9	33.9 ± 5.1	25.7 ± 17.5	40.5 ± 8.4
Nov 2010	21	53.9 ± 4.8	53.9 ± 4.8	42.8 ± 3.1	44.5 ± 10.5	12.7 ± 10.6
Dec 2010	25	53.9 ± 4.8	44.9 ± 4.4	41.8 ± 8.8	47.6 ± 6.4	10.6 ± 9.0
Jan 2011	25	52.7 ± 3.9	47.3 ± 3.9	40.4 ± 5.9	47.5 ± 3.5	12.1 ± 7.5
Feb 2011	25	49.8 ± 3.0	50.2 ± 3.0	31.0 ± 8.9	45.7 ± 2.2	23.3 ± 9.9
Apr 2011	7	50.6 ± 2.5	49.4 ± 2.5	47.5 ± 3.2	47.6 ± 1.3	4.9 ± 3.3
May 2011	8	48.5 ± 4.0	51.5 ± 4.0	49.3 ± 2.1	45.9 ± 2.4	4.8 ± 2.9
Jul 2011	25	64.3 ± 5.7	35.7 ± 4.6	22.3 ± 4.9	32.4 ± 6.9	45.3 ± 9.5
Oct 2011	22	65.0 ± 8.0	35.0 ± 8.0	39.5 ± 7.5	47.8 ± 13.1	12.7 ± 16.7
Nov 2011	19	56.8 ± 3.2	43.2 ± 3.2	28.2 ± 4.5	47.3 ± 2.9	24.4 ± 5.8
Dec 2011	22	54.7 ± 5.7	45.3 ± 5.7	28.1 ± 5.3	45.8 ± 3.4	26.1 ± 7.5
Jan 2012	25	53.4 ± 4.8	46.6 ± 4.8	38.5 ± 7.2	45.7 ± 5.2	15.8 ± 8.2
Feb 2012	25	50.5 ± 3.5	49.5 ± 3.5	32.6 ± 8.9	44.4 ± 3.3	23.0 ± 9.4
Mar 2012	25	50.2 ± 4.1	49.8 ± 4.1	46.7 ± 6.0	45.8 ± 3.3	7.5 ± 6.1

Values are given as mean ± SD. Abbreviations: w_W = wet weight, w_D = dry weight.

Hepatopancreas biochemistry

Moisture content: Moisture content ranged from 61.3 -72.4% throughout the study period. Although no clear trend is evident, highest moisture contents were recorded in autumn and winter each year, after which they declined until summer (Table 3; Fig. 7A).

Lipid content: Lipid content ranged between 22- 49.4%, with lowest values recorded in October to January/February each season, followed by an increase to peak values during autumn (Table 3; Fig. 7B).

Protein content: Protein content followed an inverse trend to lipid content (Fig. 7B). Lowest protein values of approximately 30% were recorded in July/October 2011, this was, however different in 2010 where the lowest value was found in April (39%). Highest values of more than 50% occurred during summer of both seasons.

Carbohydrate and ash content: The course of these compounds indicates a peak during summer and a low during winter. However, there were large fluctuations in individual values (Table 3).

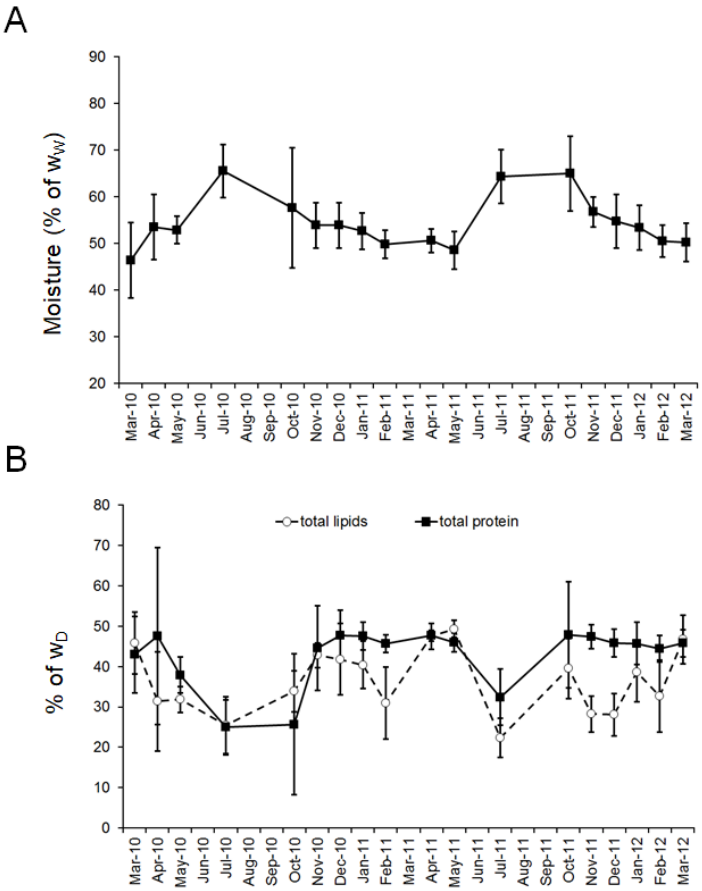

Figure 6. Seasonal course of biochemical composition of ovary samples from female *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. A) Moisture content, B) lipid- and protein contents. Values are means ± S.D., n = 7 - 30. Abbreviations: w_W = wet weight, w_D = dry weight.

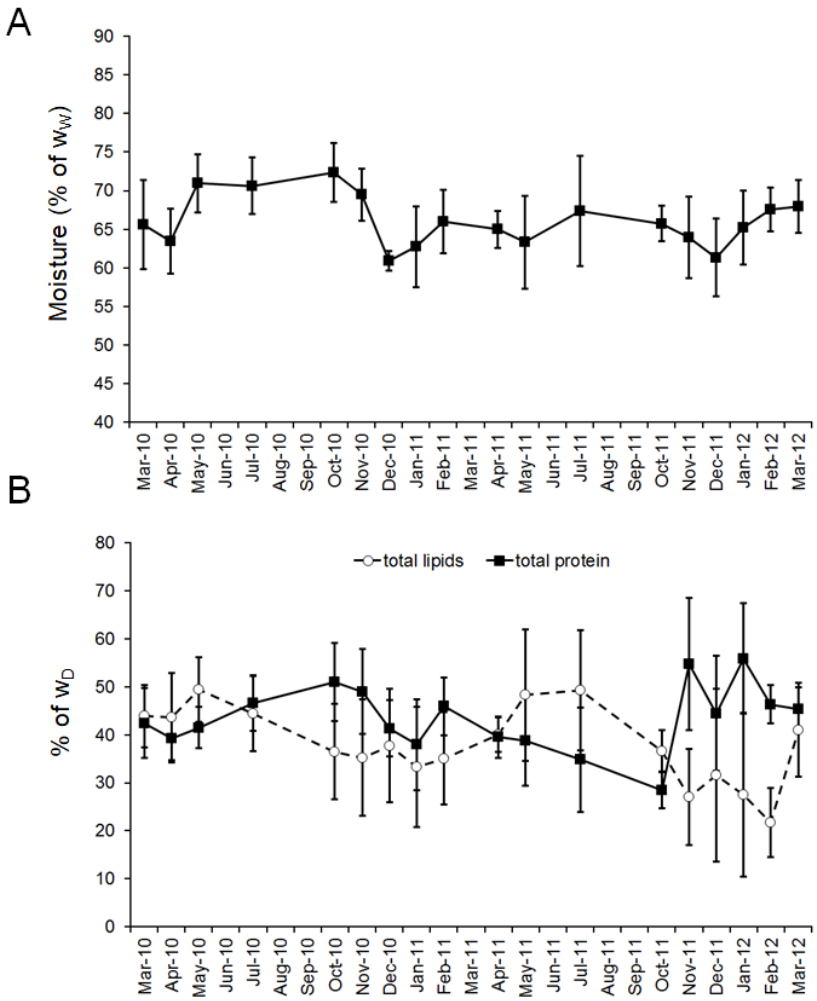

Figure 7. Seasonal course of biochemical composition of hepatopancreas samples from female *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. A) Moisture content, B) lipid- and protein contents. Values are means ± S.D., n = 7 - 30. Abbreviations: w_W = wet weight, w_D = dry weight.

Potential indicators

The biological indicators GSI, HSI and TI (Hout Bay and Olifantsbos) as well as moult stage (Hout Bay) were analyzed in relation to ovary maturation.

A sequential increase in GSI with increasing ovarian maturation stage was recorded in Hout Bay and Olifantsbos (Fig. 8A and 9A) with values in ovarian stages 4 and 5 significantly higher than in stage 1 in both areas. While the GSI values at stage 1 were similar in Hout Bay (0.8%) and Olifantbos (0.7%), the values for the more mature stages (4 and 5) were higher in Hout Bay (4 and 5.3% respectively) compared to Olifantsbos (3 and 4.1% respectively). No ovaries in stage 6 (spent) were recorded for either sampling site.

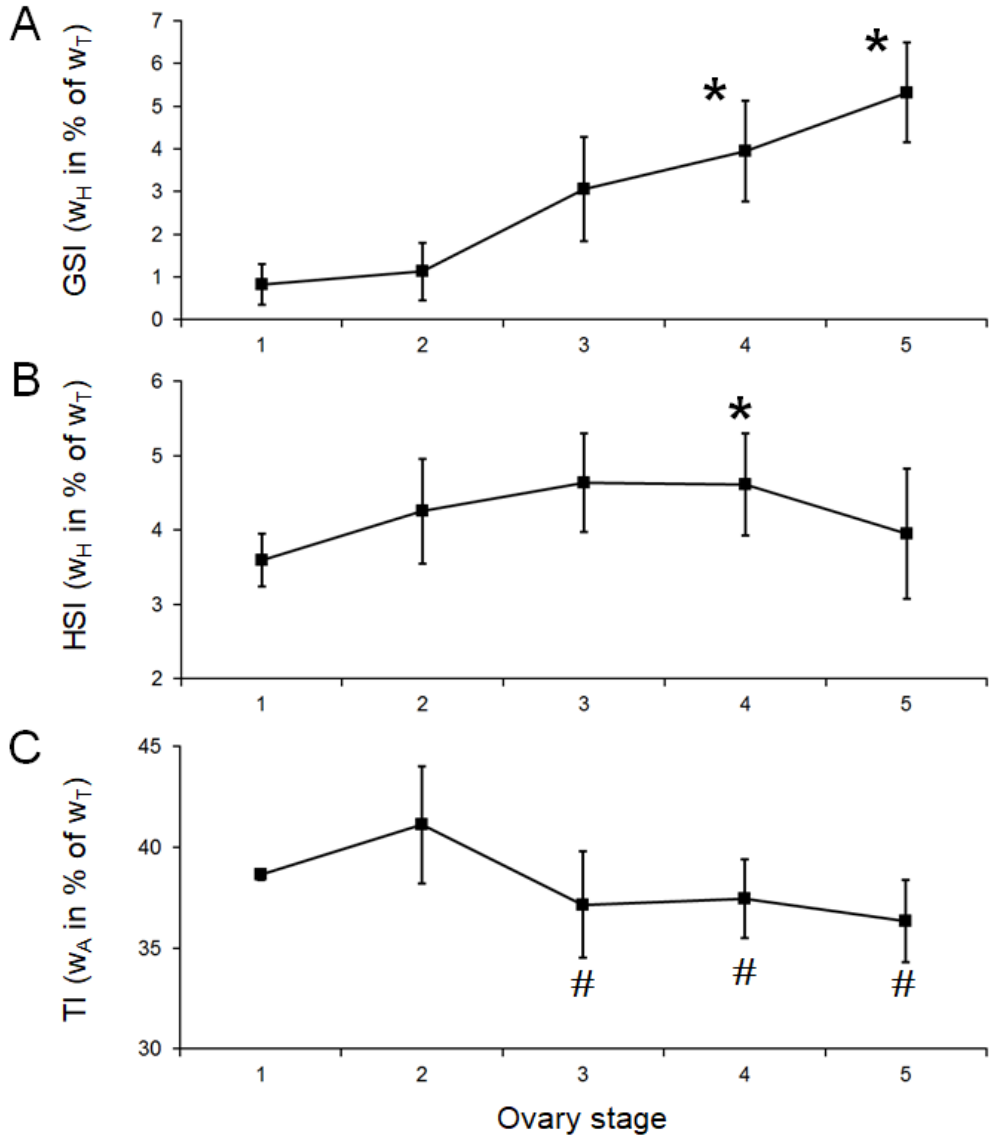

Figure 8. Variation in biological indices during ovary maturation in female *J. lalandii* from the Hout Bay area. A) GSI, B) HSI and C) TI. Data were analysed per gonad stage. Note: No lobsters with gonads at stage 6 (spent) were found in the present study. *Significant difference ($p < 0.05$) to ovary stage 1 (one-way ANOVA followed by Dunn or Holm-Sidak (B) Analysis). #Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Dunn Analysis). Values are means ± S.D., n = 3 (stage 1), n = 111 (stage 2), n = 201 (stage 3), n = 94 (stage 4), n = 15 (stage 5).

HSI in Hout Bay was lowest in stage 1 (3.6%) and increased significantly to 4.6% in stage 4, after which it decreased to 4.0% in stage 5 (Fig. 8B). In contrast, there was no trend in Olifantsbos where values were in a narrow range between 3.9 and 4.6% throughout (Fig. 9B).

The TI in both Hout Bay and Olifantsbos showed a similar trend with a slight increase from stage 1 to stage 2 (to around 40%) followed by a significant decrease to lower levels (around 36%) in the subsequent ovarian stages.

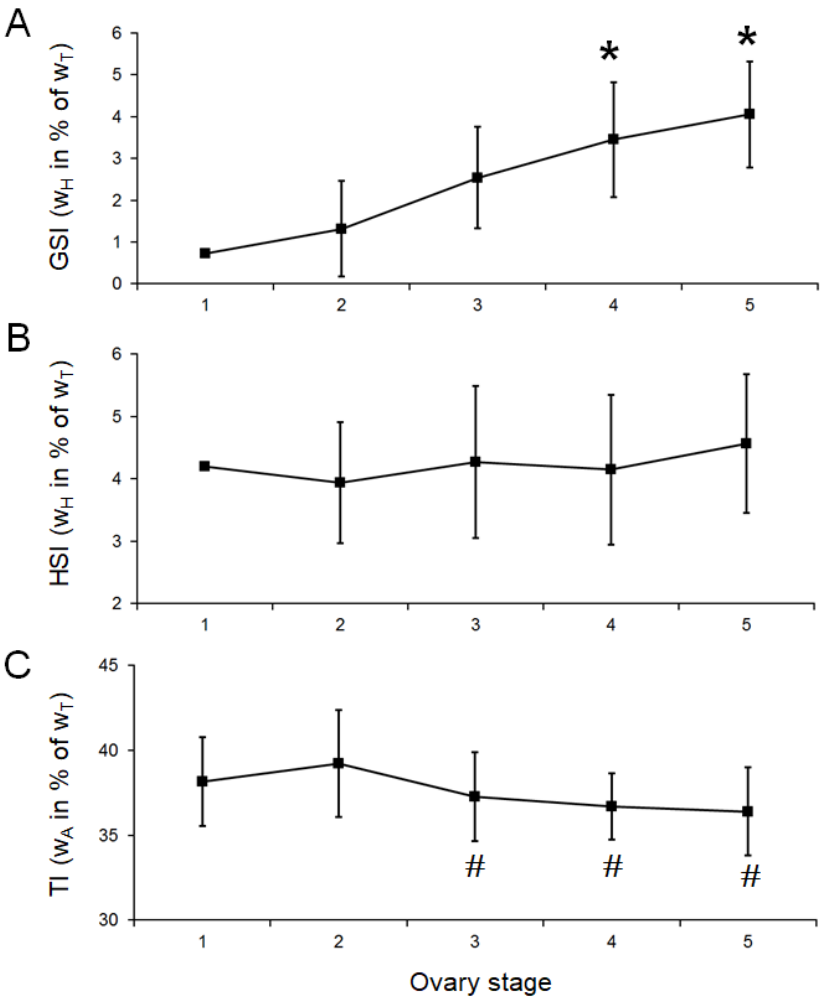

Figure 9. Variation in biological indices during ovary maturation in female *J. lalandii* from the Olifantsbos area. A) GSI, B) HSI and C) TI. Data were analysed per gonad stage. Note: No lobsters with gonads at stage 6 (spent) were found in the present study. *Significant difference ($p < 0.05$) to ovary stage 1 (one-way ANOVA followed by Dunn Analysis). #Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Dunn Analysis). Values are means ± S.D., n = 3 (stage 1), n = 87 (stage 2), n = 261 (stage 3), n = 193 (stage 4), n = 11 (stage 5).

For Hout Bay samples, biochemical parameters in ovaries and hepatopancreas were analyzed in relation to ovary maturation. Ovary moisture content declined from 67% in stage 1 to around 52 – 56% in stages 3 – 5 (Fig. 10A). Lipid content increased significantly from around 30% in stages 1 and 2 to about 40% in stage 5 (Fig. 10B). Protein content nearly doubled from 24% in stage 1 to a plateau of about 45% at stages 3 to 5 (Fig. 10C). In the hepatopancreas, moisture content declined from 72% in stage 1 to around 65% at stages 3 to 5 (Fig. 11A). Lipid content decreased from 43% in stage 1 to about 36% in stage 4 and subsequently increased to 46% in stage 5 (Fig. 11B). Protein content declined from 50% in stage 1 to a plateau of about 41 – 45% in stages 2 – 5 (Fig. 11C).

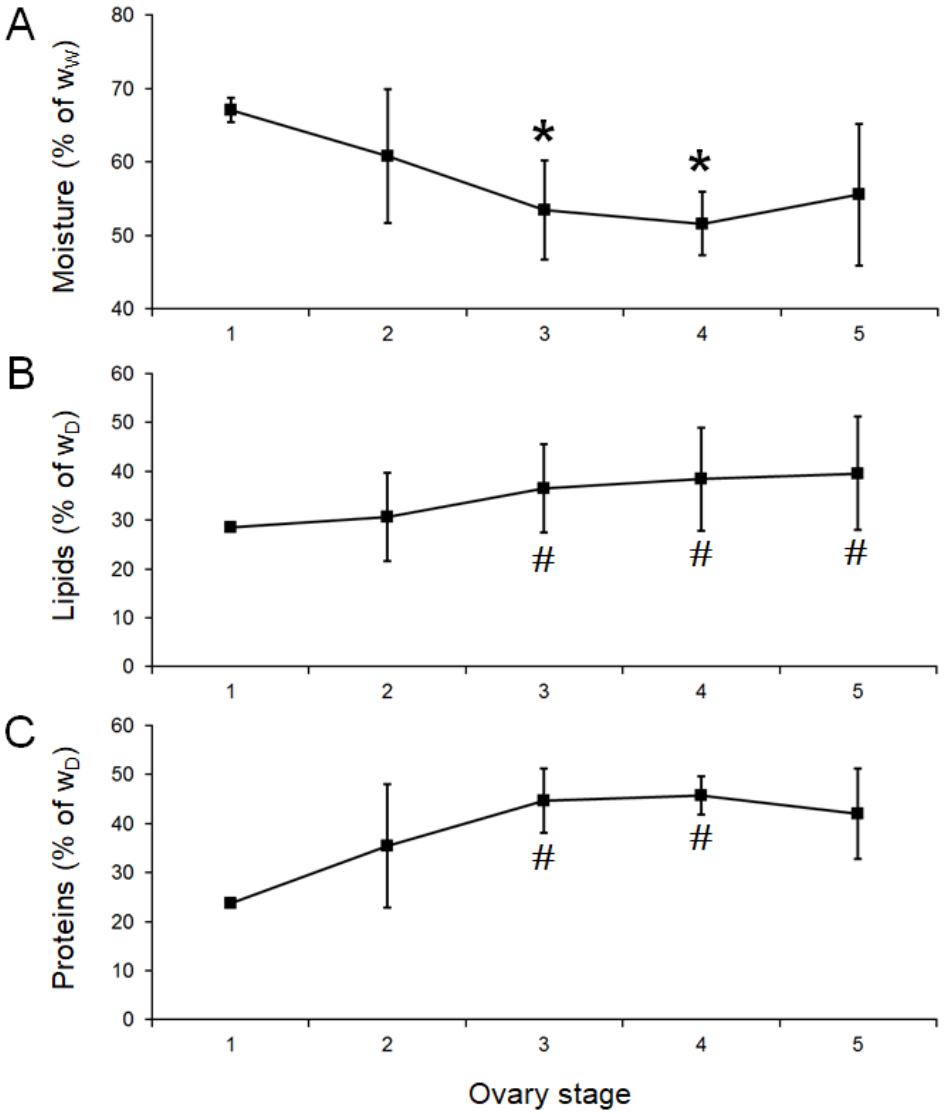

Figure 10. Levels of ovary A) moisture-, B) lipid- and C) protein contents during the respective ovary stage in female *J. lalandii* from the Hout Bay area. Analysis was carried out on lobsters from the Hout Bay sampling site only. Note: No lobsters with gonads at stage 6 (spent) were found in the present study. *Significant difference ($p < 0.05$) to ovary stage 1 (one-way ANOVA followed by Dunn Analysis). #Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Holm-Sidak (B) or Dunn Analysis). Values are means ± S.D., n = 3 (stage 1), n = 85 (stage 2), n = 192 (stage 3), n = 87 (stage 4), n = 6 (stage 5). Abbreviations: w_H = ovary wet weight, w_D = dry weight.

The biological parameters of Hout Bay lobsters were analyzed in relation to moult stage. GSI increased continuously from 2.2% in stage C (intermoult) to 3.9% in stage D_2 (premoult) (Fig. 12A), indicating the synchronicity of the two cycles. HSI showed no tend and ranged between 4.4 – 4.6% throughout the moult cycle (Fig. 12B). TI also showed little variation over the moult cycle at around 48% (Fig. 12C). Ovaries matured from a mean stage of 2.7 in stage C to around 3.3 in stages D_1 and D_2 (Fig. 12D).

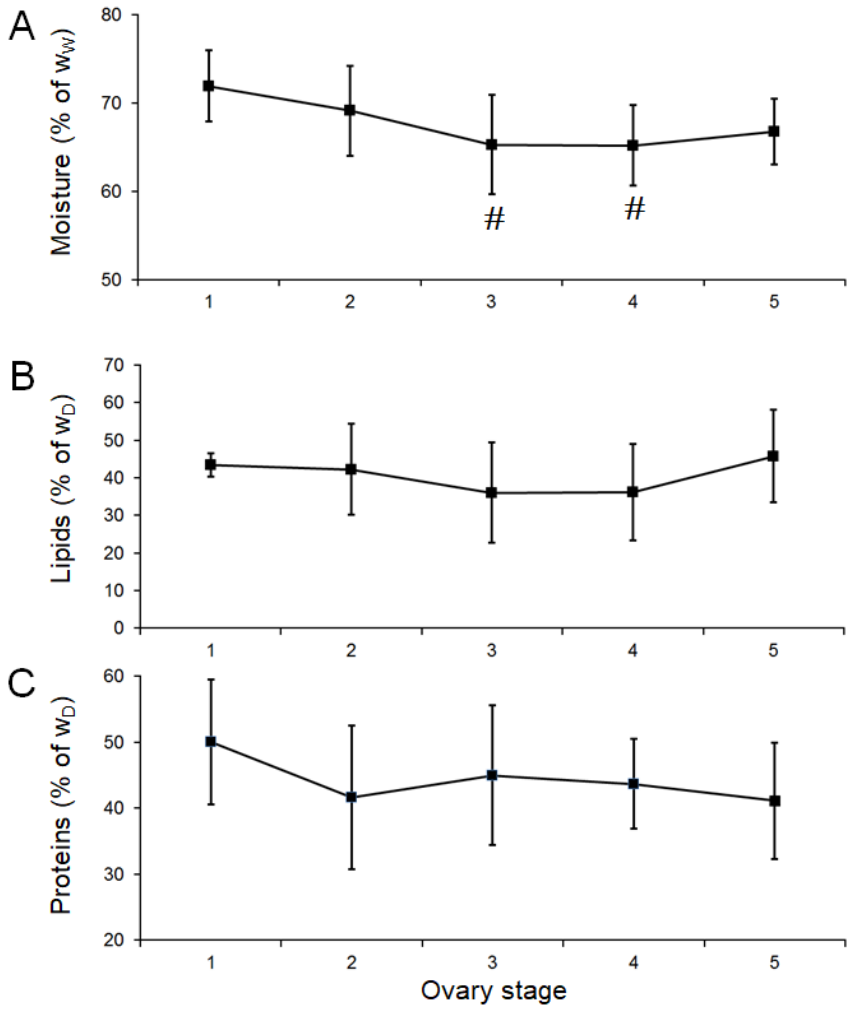

Figure 11. Levels of hepatopancreas A) moisture-, B) lipid- and C) protein contents during the respective ovary stage in female *J. lalandii* from the Hout Bay area. Analysis was carried out on lobsters from the Hout Bay sampling site only. Note: No lobsters with gonads at stage 6 (spent) were found in the present study. #Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Dunn Analysis). Values are means ± S.D., n = 3 (stage 1), n = 104 (stage 2), n = 204 (stage 3), n = 94 (stage 4), n = 15 (stage 5). Abbreviations: w_H = ovary wet weight, w_D = dry weight.

The biochemical parameters of ovaries and hepatopancreas of Hout Bay lobsters were also analyzed in relation to moult stage. Moisture content of ovaries declined continuously as moult cycle progressed: From 57% in stage C it fell to 48% in stage D_2 (Fig. 13A). In the hepatopancreas, moisture content was in a narrow range of 66 to 70% (Fig. 13B). Ovary lipid content increased slightly from 33% in stage C to 38% in stage D_2 (Fig. 13C), whereas it declined from 39% in stage C to 29% in stage D_2 in the hepatopancreas (Fig. 13D). Protein content was flat at approximately 41 to 44% in ovary samples (Fig. 13E), whereas there was a consistent increase from 42 to 50% in the hepatopancreas from stage C to stage D_2 (Fig. 13F).

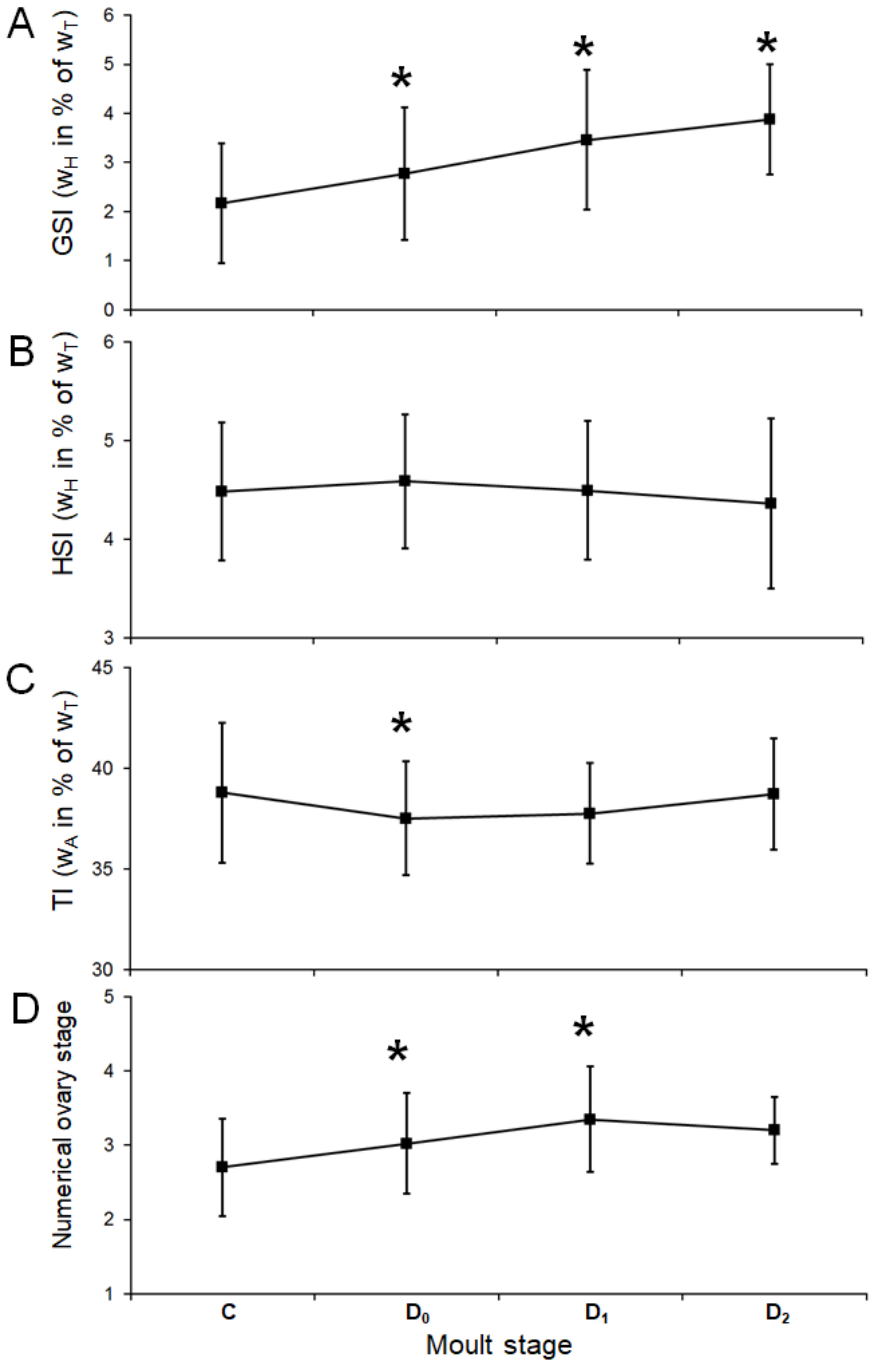

Figure 12. Levels of A) GSI, B) HSI and C) TI and D) ovary maturation stage during the respective moult stage in female *J. lalandii* from the Hout Bay area. Analysis was carried out on lobsters from the Hout Bay sampling site only. *Significant difference ($p < 0.05$) to intermoult (stage C) (one-way ANOVA followed by Dunn Analysis). Values are means ± S.D., n = 113 (stage C), n = 164 (stage D_0), n = 67 (stage D_1), n = 5 (stage D_2). Abbreviations: w_T = total wet weight, w_D = dry weight.

Analysis of the interrelationship of protein- and lipid content in the ovaries revealed a positive regression (Fig. 14A), whereas there was an inverse relationship in the hepatopancreas (Fig. 14B).

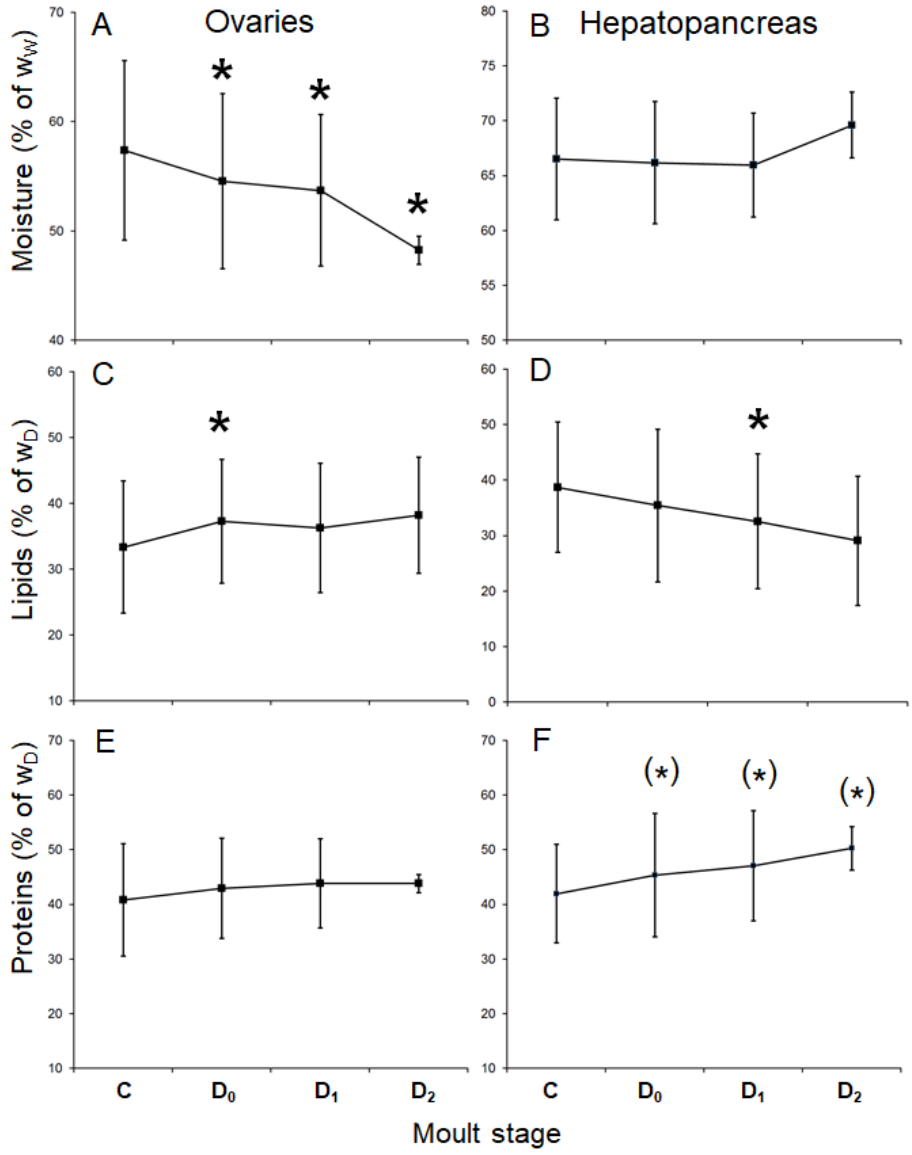

Figure 13. Levels of moisture content in A) ovaries and B) hepatopancreas, lipid content in C) ovaries and D) hepatopancreas, protein content in E) ovaries and F) hepatopancreas during the respective moult stage in female *J. lalandii* from the Hout Bay area. Analysis was carried out on lobsters from the Hout Bay sampling site only. *Significant difference ($p < 0.05$) to intermoult (stage C) (one-way ANOVA followed by Dunn Analysis). Values are means ± S.D., n = 98 (stage C), n = 152 (stage D_0), n = 66 (stage D_1), n = 5 (stage D_2). Abbreviations: w_T = total wet weigh, w_D = dry weight.

Female lobsters from the same area were, with few exceptions, in a relatively narrow size range and hence comparable. The absolute lipid weight per ovary was therefore used as a factor to estimate the total amount of lipids available for reproduction and as a possible predictor for male growth. First, a seasonal course was constructed for this parameter (wet weight lipid concentration multiplied by ovary wet weight): highest values occurred in autumn of each calendar year (above 2 g) and lowest (below 0.5 g) in austral winter (Fig. 15).

The highest values recorded for each sampling year were relatively similar: 2.4 g in March 2010, 2.3 g in May 2011 and 2.5 g in March 2012 (Fig. 15; Table 4). Lipid weight per ovary is mainly driven by organ size and not lipid concentration: GSI peaked at a similar time as the lipids per ovary (Figs. 4A, 15) whereas lipid concentration is at a high level from about October/November to March - May (Fig. 6B). In the latter months, the last sampling before spawning took place; data from this maximum for each year were therefore further considered for their potential to predict growth in the subsequent male moult. However, relevant comparable parameters (GSI, lipid per w_W, lipid per w_D, lipid per ovary) were very similar for all three calendar years (Table 4).

Table 4. Select biological and biochemical data from the peak months of March 2010, May 2011 and March 2012 from the Hout Bay fishing area.

	n	Male growth	w_T	w_O	GSI	stage	Lipid per dry weight	Lipid per wet weight	Lipid per ovary
		mm	g	g	% w_T	numerical	% w_D	% w_W	g
Mar 2010	16	2.7 ± 1.2	189 ± 25	7.7 ± 2.7	4.0 ± 1.2	3.2 ± 0.4	58.1 ± 7.7	31.2 ± 6.3	2.4 ± 1.0
May 2011	25	3.4 ± 1.8	201 ± 20	7.6 ± 3.9	3.8 ± 2.0	3.6 ± 1.0	61.6 ± 2.6	31.7 ± 2.7	2.3 ± 1.3
Mar 2012	25	2.8*	208 ± 26	8.6 ± 2.4	4.2 ± 1.2	3.8 ± 0.5	58 ± 7.5	29.0 ± 3.9	2.5 ± 0.9

Values are given as mean ± SD. Abbreviations: w_T = total wet weight, w_W = wet weight, w_O = ovary wet weight, w_D = dry weight, w_H = hepatopancreas wet weight, HSI = Hepatosomatic Index. *No value determined. Estimated from average differences in growth increment of all other fishing seasons. No tag returns due to unavailability of own ships and no fishing in the area by industry (see Chapter 3).

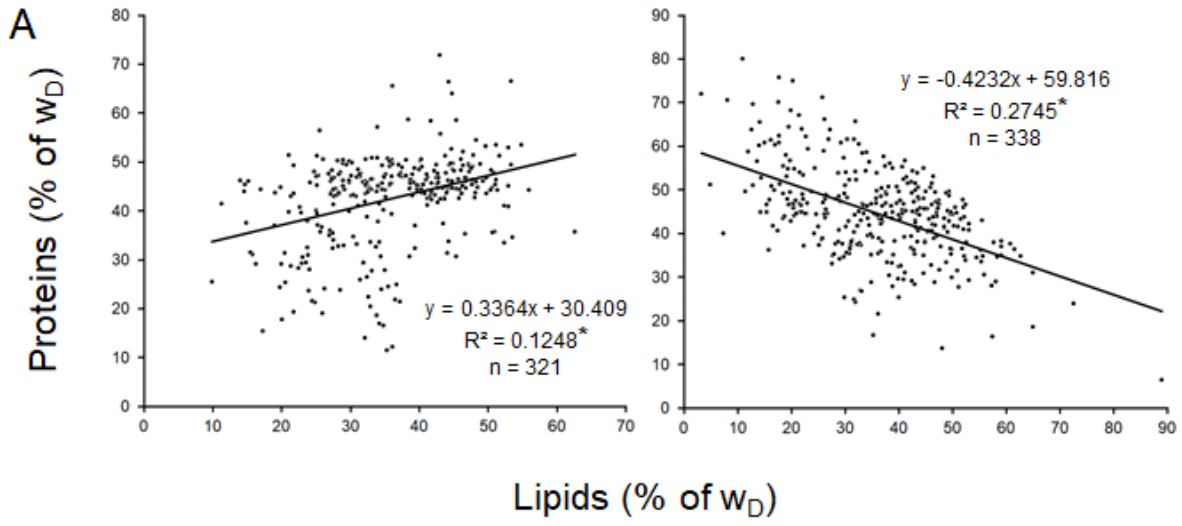

Figure 14. Interrelationships of A) ovary lipid and protein concentration and B) hepatopancreas lipid and protein concentration from female WCRL analysed from the Hout Bay sampling site. *Significant correlation ($p<0.05$) of linear regression (ANOVA). Abbreviation: w_D = dry weight.

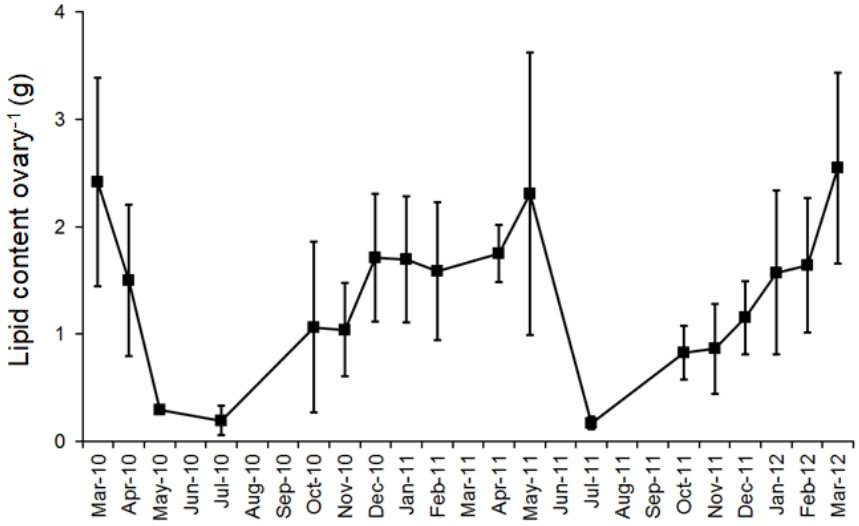

Figure 15. The course of the lipid content per ovary of female *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. Values are means ± S.D., n = 7 – 27

Reserve partitioning between growth and reproduction

In female lobsters, the hepatopancreas and ovaries are the two main metabolite reservoirs, and these reserves must be apportioned to either growth or reproduction. Therefore, the sum of stored lipids and proteins in both organs was first determined. The sum of lipids reached a maximum (up to 3.6 g) in late summer/early autumn and a minimum (1.3 – 2.0 g) in winter each year (Fig. 16A). The trend is similar for the sum of proteins in both organs although not as pronounced (Fig. 16B). In a second step of analysis, the share of both organs for each metabolite throughout the sampling period was determined. For lipids, the share of gonads is lowest in winter (14 – 24%) and highest in late summer/autumn (55 – 67%) (Fig. 17A), whereas the share of gonad proteins follows a similar trend (Fig. 17B).

4.4 Discussion

Adult/sexually mature male and female lobsters moult once a year, with moulting and reproductive cycles tightly coupled (Pollock, 1986). Adult females usually moult in winter, mate with hard-shelled (intermoult) males and then extrude their eggs to become egg-bearing females some weeks later (Cockcroft, 1997). As a result, accumulation of reserves in male hepatopancreas and in developing female gonads and hepatopancreas occurs over the same period (Heydorn, 1969), and, as genders are not spatially separated during this period, similar environmental conditions would be experienced by both sexes (Cockcroft, 1997). The reserves accumulated in the male haptopancreas of *J. lalandii* have been shown to be linked with male growth (Chapter 3; Cockcroft, 1997) and a positive association between higher egg production in females and elevated male growth was described by Melville-Smith et al. (1995). This

chapter examines the reserve accumulation in tail, hepatopancreas and ovaries of female lobsters from a low growth rate (Olifantsbos) and a high growth rate (Hout Bay).

Previous findings of biological and biochemical parameters in *J. lalandii* and palinurids in general were confirmed by the present study: maximum seasonal HSI values of about 5 – 5.2% measured by the present study are similar to those found for *J. lalandii* males (Chapter 3, Cockcroft, 1997), but higher than those for similar-size females (<3.6%) from a study conducted in 1994 (van Rooy, 1998) and in (much larger) female *J. edwardsii* (Smith et al., 2004). Maximum seasonal GSI values of 3.9 – 4.6%, however, were in a similar range as reported previously for *J. lalandii* (van Rooy, 1998) and *J. edwardsii* (Smith et al., 2004). The TI of 33 – 42% was generally higher and seasonally more variable than in males (33 – 34%) from the same period and areas (Chapter 3). The TIs from females from the two sites were in a similar range (Figs. 1A and 2A). Hepatopancreas lipid contents recorded here (reaching close to 50% w_D) are at the same level as in the respective males (Chapter 3, Cockcroft, 1997). These values are lower, however, than levels of 70-75% w_D for female *J. edwardsi* (Smith et al., 2004). Hepatopancreas protein levels recorded here are also similar to those reported for male *J. lalandii* (Chapter 3).

Seasonal variations in female body parameters are linked to moult- and reproductive cycles, however, they are not as obvious as in males. Relative hepatopancreas size (HSI) in both fishing areas, although low in austral winter (Figs, 1C, 2C, 12B), does not indicate a seasonal trend in reserve accumulation. This contrasts with males in which HSI peaks shortly before moult after which it expands again (Cockcroft, 1997; Chapter 3). A similar lack of accumulation in female hepatopancreas was reported by van Rooy (1998). The low values in winter are probably related to the female moult period, as indicated by the low GSI at the same time. This is corroborated by a decline in hepatopancreas lipid levels in the Hout Bay area (Fig. 7B), for which composition was determined. Female spawning follows some weeks after moult and may also contribute to this low level. Throughout the season, female hepatopancreas size, as judged by HSI, is about 7-8% smaller than in males at both fishing areas (Table 2, Chapter 3). In contrast to HSI, both, annual progression of relative gonad weight (GSI) and gonad stage are clearly seasonal with a low in the spawning season in both fishing areas (Figs. 4AB and 5AB).

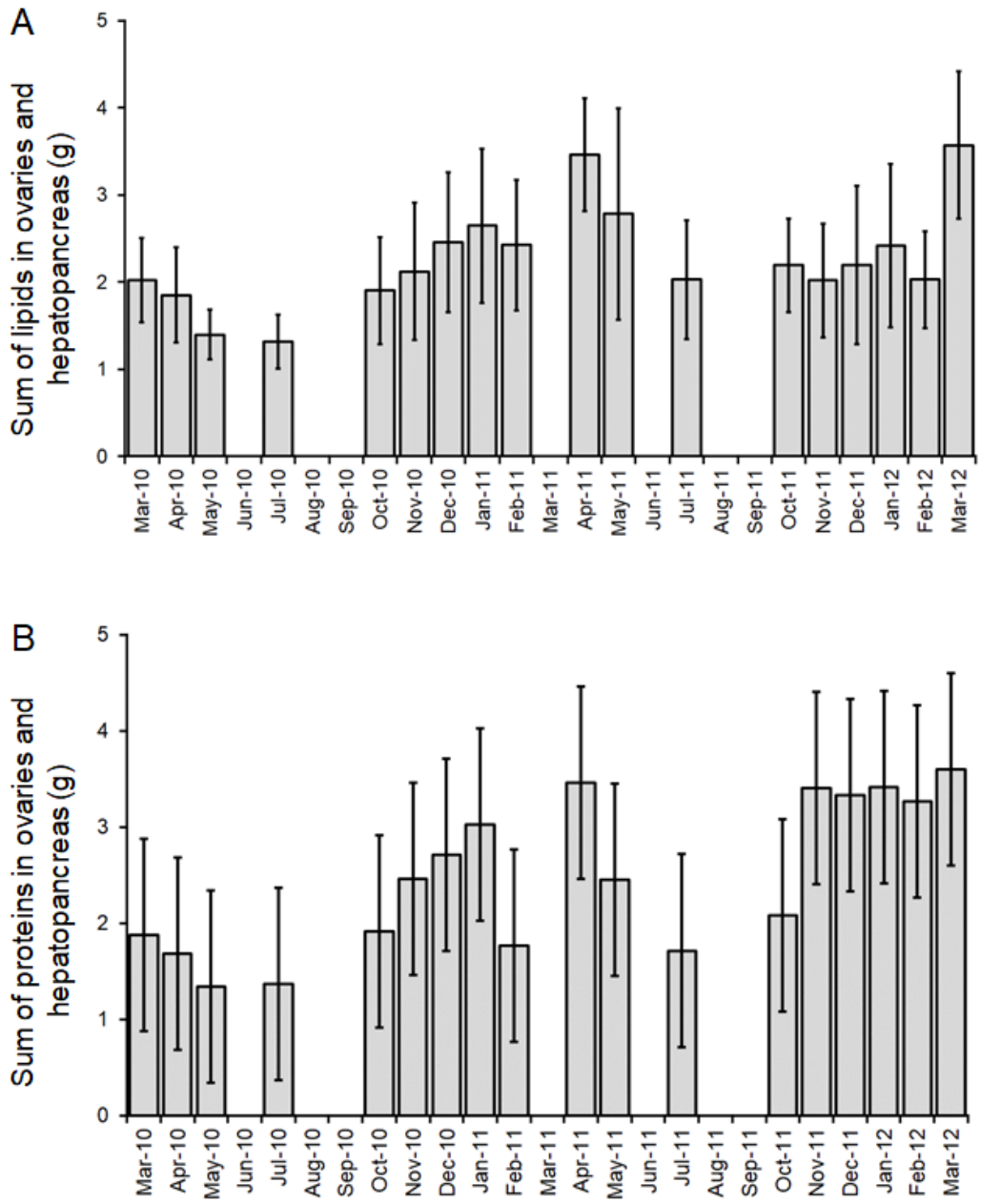

Figure 16. Total amount of A) lipids and B) proteins in both hepatopancreas and ovaries of female *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. Values are means ± S.D., n = 7 - 27.

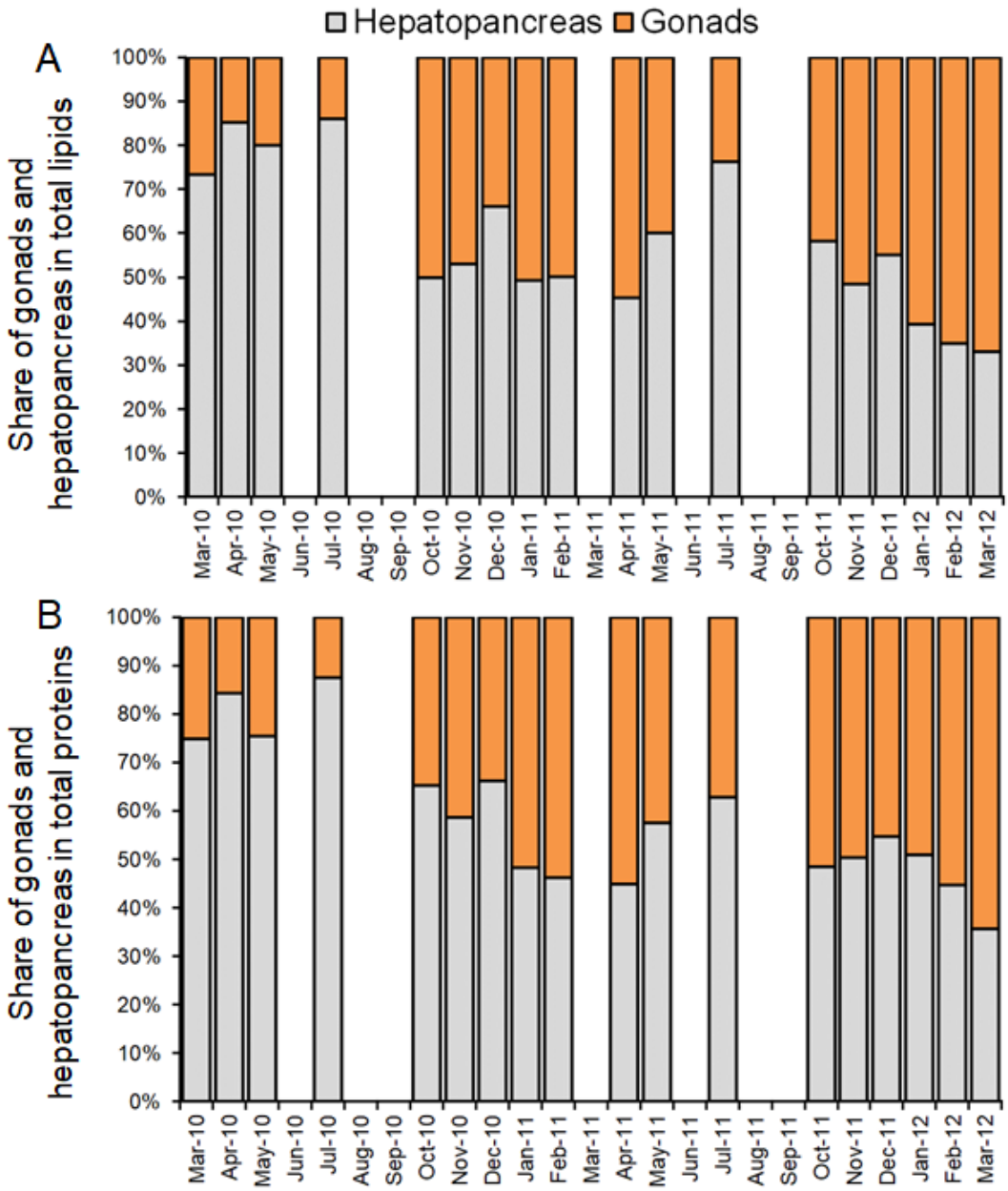

Figure 17. Share of A) lipids and B) proteins in hepatopancreas and ovaries of female *J. lalandii* caught at the Hout Bay sampling site (”Knol”) during the period from March 2010 to March 2012.

This corroborates with the trend reported for females from the same fishing grounds in 1994 (van Rooy, 1998). Ovary protein concentration and, to a lesser extent, lipid concentration in the ovaries reach a low that coincides with the low of the numerical gonad stage indicator and increase steadily from there to reach plateaus over the end of the sampling years (Fig. 6B). In contrast to female and male hepatopancreas, protein- and lipid concentrations in the ovaries showed a positive regression (Fig. 14A,B), indicating the accumulation of vitellogenin. Compared with males, the larger relative size of the tail (see above) may indicate a function of the lipid- and protein rich abdominal muscle as an additional storage site for reproduction (see Chapter 2). In addition, despite the lack of a conclusive seasonal trend that would indicate reserve fluctuation in the abdominal muscle, there is a decline in TI from ovary stage 2 onwards in both sampling areas (Figs. 8C and 9C). This may indicate an involvement in ovary maturation. At the Hout Bay sampling site, there is also a decline in TI during the moult cycle (Fig. 12C), namely from intermoult (C) to early premoult (D_0). So, involvement in both cycles may prevent an obvious seasonal trend in Fig. 8C and Fig. 9C.

Historically, the moult stage of *J. lalandii* was determined using the subjective shell hardiness index method (see Chapter 3). Setagenic analysis of pleopods was therefore included in the present study for females from the Hout Bay fishing area to provide a more accurate measure of moult stage. The challenges faced in exact moult stage determination were similar to those reported for male lobsters (see Chapter 3). Large variation and overlap of stages at almost all times of the season were found (Fig. 4). For instance, lobsters in stages C and D_0 were recorded for every month sampled. As a result of these challenges, capturing the moult process numerically is not very conclusive (Fig. 4C). In addition, some months of sampling were lacking for logistical reasons (see Chapter 3). However, the obtained data were useful in analyzing biological events during the growth- and reproductive cycles. In the course of the moult cycle, GSI and numerical ovary indicator increase as they develop in parallel for a long period (Fig. 4A,B and 5A,B). There is no change in HSI and a small drop in TI that coincides with the onset of ovarian increase, by both stage and GSI (Fig. 12A,D). Moisture declines in the ovaries as moult progresses, but not in the hepatopancreas (Fig. 13A,B). In the ovaries, a slight accumulation of lipids and proteins occurred (Fig. 13C, E), whereas in the hepatopancreas, lipids decline substantially, concomitant with an increase in proteins (Fig. 13D,F). Both reach plateaus in premoult stages D_0 and D_1. No lobsters in stage $D_{3\text{-}4}$ (~10 days long, Marco, 2012) were caught in the present study, probably because there was no sampling

during August and September. However, most likely ovaries continue to expand in stage D_{3-4} and beyond moult.

Although not very clear from the seasonal progression of parameters, details of the ovary maturation cycle become more obvious when analyzed per ovarian stage. As maturation progresses, ovary size (GSI) increases consistently in females from both fishing areas (Fig. 8A and 9A), whereas hepatopancreas size (HSI) from Hout Bay females increases until gonad stage 4 after which it declines (Fig. 8B). However, this is not the case in females from the Olifantsbos area (Fig. 9B). Clear and concomitant ovarian accumulation of proteins and lipids can also be observed during the sequence of the ovarian cycle (Figs 10B,C). No lobsters in ovarian stage 6 (spent) were caught in the present study but previous research showed an almost complete depletion of lipids and proteins and a sharp decline of relative gonad size in this stage (van Rooy, 1998). From a relatively high level at ovary stage 2, TI declines (3 - 5% of total weight) towards stage 5 in females from both fishing areas (Fig. 8C and 9C). This decline seems small but considering the relatively large body share of the tail, this amounts to 6 - 10 g in a 200 g lobster. For a substantial period, ovarian maturation, and moult preparation progress simultaneously. As a result, numerical moult stage indicator increases consistently until stage 5 in Hout Bay (Fig. 8D). As ovaries mature, water content (moisture) is replaced by the accumulating lipids and proteins to form vitellogenin (Figs. 10). In the hepatopancreas, moisture also declines with ovary maturation (Fig. 11A), however, there is little change in lipid concentration and a small decline in protein content (Fig. 11B,C).

The dynamics of metabolic reserve accumulation in decapod ovaries and hepatopancreas are interlinked (Harrison, 1990). The accumulation of proteins and lipids is a result of vitellogenesis (production of vitellogenin and lipoprotein and storage by oocytes) in the ovaries. However, a contribution of the hepatopancreas to this process is likely with subsequent transfer to the ovaries via the haemolymph (Harrison, 1990). The exact details of partitioning of reserves between reproduction and growth, i.e., ovaries and hepatopancreas, were only studied in a limited number of crustacean species. In the present study, the partitioning of overall reserves and apportioning to hepatopancreas and/or ovary (i.e., growth and/or reproduction) were analysed from the collected data. In decapods, five categories were identified as to if/how reproduction is coordinated with the moult cycle (Raviv et al., 2008). As the moult and reproductive cycles in *J. lalandii* are tightly coupled, they would fall into category *d* of Raviv and co-authors' review. Females are in early intermoult when mating and extruding eggs (Heydorn, 1969) so that moult is not in the way of or competing with spawning

and subsequent egg-bearing. This sequence has consequences for the partitioning of reserves between moult and reproduction. Previously, three ways of reserve partitioning between the two have been categorized: 1) Metabolic reserves are stored in the hepatopancreas from where they are mobilized for ovary maturation, leading to cyclical variations in hepatopancreas- and ovaries reserves through the breeding season. This was shown in some crabs and prawns (Kyomo, 1988; Castille and Lawrence, 1989). 2) No storage occurs in the hepatopancreas, and resources are directly apportioned to growth and reproduction. This was observed in some crab- and prawn species (Heath and Barnes, 1970; Pillay and Nair, 1973; Castille and Lawrence, 1989; Hasek and Felder, 2005, 2006). 3) Reproduction is prioritized over growth during limited food availability in that only a surplus is apportioned to growth and no storage in the hepatopancreas occurs. This is necessary because reserve use for reproduction and growth are antagonistic (Nelson, 1991). Such prioritization was found in isopods and hermit crabs (Nelson, 1991). In female *J. lalandii* in the present study, the total available amount of lipids and proteins in both hepatopancreas and ovaries accumulates until late summer/early autumn after which it declines to a minimum in winter (Fig. 16A,B). This indicates reserve accumulation for both growth and reproduction. However, from these results, it is impossible to estimate as to how these reserves are partitioned between the two processes. When analyzing the share of each organ in the sum of available metabolites, a clear increase of the metabolite share of the gonads from winter to the following autumn becomes visible (Fig. 17A,B). In the case of gonad lipids, the share more than doubles (Fig. 17A). Most metabolites are therefore apportioned to the gonads and no clear “moult peak” in the hepatopancreas share is visible. It is therefore likely that metabolites accumulate in ovaries but not in the hepatopancreas of *J. lalandii* during ovarian maturation. In addition, the size of the ovaries increases whereas hepatopancreas size remains relatively unchanged. This confirms earlier findings by van Rooy (1998). There are hence no reserves accumulated in the hepatopancreas first and transferred to the ovaries later. *J. lalandii* can therefore be placed into category 2). As known from previous research, female *J. lalandii* sacrifice growth for reproduction when food is scare (Cockcroft, 1997). Hence, they can additionally be placed into category 3). In the closely related palinurid *J. edwardsii*, the situation seems similar: There is indirect evidence that lipid demand of ovarian maturation is to a large extent met through dietary intake and not only (or not at all) from the hepatopancreas (Smith et al., 2004). However, in *J. edwardsii*, starvation leads to a reduction in ovary size and lipid content (Smith et al., 2004). The role and contribution of the abdominal muscle in both growth and reproduction is not conclusive from the present study. However, there are indications that it may be involved in providing reserves for ovary maturation females.

The positive relationship of male growth and female fecundity that was previously shown (Melville-Smith et al., 1995) results from built-up of metabolic reserves in male hepatopancreas and female gonads during the same period in *J. lalandii* (Heydorn, 1969; Cockcroft, 1997), implying that more eggs are produced in areas where male somatic growth is fast (Beyers and Goosen, 1987). Optimal reserve built-up in the ovaries should therefore result in higher fecundity and, in turn, should indicate higher somatic growth of male counterparts. To qualify as potential growth predictor, aspects of this built-up should show distinct variation over moult- or ovary maturation cycles or over the season (Cockcroft, 1997). Due to the potential involvement of the hepatopancreas in ovary maturation (Harrison, 1990), the hepatopancreas should be included in such analyses. In the present study, parameters of the hepatopancreas (HSI, lipid content) were not as clearly seasonal as in males, displaying much less defined peaks (Figs. 1C,2C). In contrast to the hepatopancreas, aspects of ovary maturation were much clearer: GSI, ovary stage, moisture-, lipid- and protein contents were all seasonal (Figs. 4-6). Some aspects of tail (abdomen) size also showed some seasonality; however, a clearer trend was observed in the tail weight/CL (Fig. 1B and 2B) ratio rather than in the TI (Figs. 1A and 2A). The course of the numerical moult stage indicator (Fig. 3C) was less defined than in males due to large differences in the moult stage the individual females were in (Fig. 4). Ovary data was therefore analyzed further. Lowest values in GSI and numerical gonad stage were detected in June of 2010 and 2011 (Figs. 5A,B, 6A,B), indicating that sampling had taken place immediately after annual spawning. Reserve accumulation should therefore peak shortly before this event. In the study period, the lipid peak (almost coinciding with the protein peak) in March 2010, May 2011 and 2012 were identified as potentially useful (Fig. 6B). They indicated the point of maximum accumulation for reproduction. Subsequently, data from these months were analyzed separately in more detail (Table 4). However, all these data are highly variable at most sampling points, indicated by high standard deviations. Moreover, the seasonal course of the parameters (and therefore peaks) shifts to a certain extent every year. This is in addition to the different size of female lobsters obtained in each year (Table 4), which depended on availability (it was much more difficult to collect sufficient females of the desired size than males). The highly seasonal ovary size and lipid content were therefore combined in the search for a more robust value. The total amount of lipid per organ (ovary) available for reproduction was obtained in this way. It displayed a clear seasonal trend (Fig. 15), a prerequisite for suitability as an indicator (Cockcroft, 1997). Due to this seasonality, this parameter seems to have strong potential to predict female fecundity and possibly male growth in subsequent spawning and male moult, respectively. However, limitations of the sampling strategy and

numerous other problems during the collection campaign most likely prevented a more accurate and conclusive analysis. For example, due to unavailability of ships time, large gaps of several months occurred. This was suboptimal because biological events, such as peak reserve accumulation, moult and spawning were spread over several months individually and show a considerable degree of annual fluctuation.

4.5 Conclusion

The present study confirmed some seasonal accumulation trends of metabolic reserves in gonads and hepatopancreas of female *J. lalandii*. Analysis on the partitioning of reserves between gonads and hepatopancreas during ovarian maturation revealed little or no reserve accumulation in hepatopancreas prior to peak ovary reserve accumulation. The study could not provide robust indicators for predicting female growth increment in the subsequent moult. However, average relative ovary size (GSI) was consistently higher in the high growth fishing area and the amount of lipids per ovary is highly seasonal. These are potential directions for future research in this regard.

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Chapter 5

Lipid metabolism during the accumulation phase in hepatopancreas and ovaries of mature *Jasus lalandii*

Abstract

Biological body parameters, including stage in moult- and ovarian cycles, and biochemical organ composition of mature male and female *Jasus lalandii* of commercial size (males) were analysed during the period of rapid reserve accumulation (austral spring to autumn) that was previously identified in a fast growth fishing area. Results confirmed parallel reserve accumulation in male hepatopancreas for growth and in female ovaries for reproduction during this period. Results of the present chapter confirm parallel reserve accumulation in male hepatopancreas and female ovaries. Moreover, whereas total fat- and cholesterol contents in male hepatopancreas and female ovaries steadily increase, fatty acid (FA) composition is stable in male and female hepatopancreas as well as in ovaries. Principal FA in male and female hepatopancreas as well as in ovaries included $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$, which comprised ~70% of all quantified FA. Changes in male hepatopancreas are associated with preparation for moult (i.e. growth) whereas in females, changes in the ovaries indicate maturation and preparation for spawning (reproduction). The FA profiles in all analysed organs remain relatively unchanged during both moult- and ovary maturation cycles, indicating that food availability in the fishing area is unchanged and optimal for growth and reproduction of *J. lalandii*. Moreover, at any time point, females had substantially more fat available than males, most likely indicating that same-size females have a higher food consumption rate and that the area provides sufficient food for optimal male growth.

Keywords: Growth, reproduction, hepatopancreas, ovaries, fat, fatty acids, reserve accumulation

5.1 Introduction

Moult and reproduction in palinurid decapods are powered by metabolic reserves deposited in the hepatopancreas and female gonads (Chapter 2). The hepatopancreas (or digestive gland), is a central organ in the biology of decapods and serves vital metabolic functions such as enzyme synthesis and secretion, uptake and storage of nutrients and their precursors, synthesis and catabolism of storage metabolites, excretion, provision of material and energy for moult and growth (Gibson and Barker, 1979). Relative hepatopancreas size and its lipid levels respond rapidly to changes in physiological and environmental parameters. Lipid depots are used up quickly in response to environmental and physiological changes such as moult (Cockcroft, 1997) and dietary stress (Smith et al., 2004). In male *J. lalandii*, the hepatopancreas provides energy and metabolites for somatic growth (Cockcroft, 1997, Chapter 3) which accumulate between the annual moulting events (Cockcroft, 1997). In females, the ovaries become an additional centre for lipid metabolism during ovarian maturation. Lipids accumulate together with proteins and the Gonadosomatic Index (GSI) increases (Harrison, 1990). In female J. lalandii, lipids and proteins in ovaries accumulate until spawning and the GSI increases concomitantly (van Rooy 1998; Chapter 4). The synchronization of male and female life cycles, lipid- and other metabolic reserves in the male hepatopancreas and female gonads build up during the same period in *J. lalandii* (Cockcroft, 1997) is supported by the positive relationship between male growth and female fecundity found by Melville-Smith et al. (1995).

In Chapters 3 and 4 the reserve accumulation during two the moult and reproductive cycles were examined from two areas, one characterised by slow male growth and the other fast somatic growth. In this chapter, the period of rapid reserve accumulation in both the male hepatopancreas and female gonads (austral spring to autumn) was selected to do more detailed analysis including changes in lipid composition and the Fatty Acid (FA) profiles in the hepatopancreas and ovaries. There is little specific information on this topic for hepatopancreas and ovaries of palinurids (Chapter 2). Composition changes in lipids and FA during ovary maturation, do, however, occur in some crabs (Mourente et al., 1994). It was shown though, that ovarian maturation and reserve build-up depend on food supply. Starvation leads to reduction of gonad index (GSI) and lipid content in *J. edwardsii* females (Smith et al., 2004). In addition, starvation and different (artificial) diets can alter the proportion of some FA in hepatopancreas and tail muscle of female *J. edwardsii* (Smith et al., 2004). The aim of this chapter was to investigate changes in storage organ parameters as well as lipid composition during the accumulation phase of male and female hepatopancreas and female gonads from the

high-growth fishing area and relate them to growth- and reproductive cycles. A more detailed examination of the changes in lipid accumulated in both organs could provide vital information in understanding the source and pathways of these essential compounds in this species as well as on the partitioning of metabolic reserves between female growth and reproduction.

5.2 Materials and methods

Mature *Jasus lalandii* male (63 – 86 mm carapace length, CL) and female (64 – 86 mm CL) rock lobsters were caught from October 2014 to April 2015 at Hout Bay ("Die Knol"; 34º04'S, 18º20'E)(Figure 1). The lobsters were captured using the research vessel RV "Ellen Khuzwayo" and standard commercial fishing gear (Pollock, 1986) at depths of approximately 80 to 200 m. After capture, Total wet weight (w_T) was determined using a digital balance and CL measured from rostral tip to posterior edge of carapace using Vernier callipers. Animals were subsequently sacrificed and dissected. Abdominal (tail) weight (w_A) was determined after dissection, whereas hepatopancreas and gonad were transferred into pre-weighed vessels. Ovary stage was recorded immediately. Samples were immediately blast-frozen aboard immediately and later transported to freezers at the laboratory where they were kept at -40 °C for later biochemical analysis and determination of weight. They remained sealed from air in a plastic vial to prevent auto-oxidation of the lipids in the sample. For determination of moult stage, a pleopod was sampled from each lobster, which was individually placed in an Eppendorf tube for storage at -25 °C.

Somatic indices, i.e. hepatosomatic index (HSI), tail index (TI) and gonadosomatic index (GSI) were calculated as described in Chapter 4.

The stage of individual lobsters in the moult cycle was determined via microscopic analysis of setagenic features of pleopods as outlined in Marco (2012) for adult *J. lalandii*. Briefly, the distal section of the pleopods were floated on a cavity slide, covered with a cover slide, and examined with a compound eclipse Ni microscope (Nikon, Japan). The moult stages of *J. lalandii* are divided into stages AB (post-moult), C (intermoult), D_0 - $D_{3\text{-}4}$ (pre-moult)(Marco 2012). The various substages of premoult stage D_1 were lumped together for simplicity and all moult stages were later coded as numerical values for analytical purposes (C = 1, D_0 = 2, D_1 = 2.5, D_2 = 3).

Table 1. Biological data of male and female *J. lalandii* caught from October 2014 to April 2015.

Month:			Oct 2014	Dec 2014	Jan 2015	Feb 2015	Apr 2015
Males							
n			20	25	25	25	25
Carapace length	CL	mm	71.5 ± 1.5	70.8 ± 3.8	72.2 ± 2.0	73.6 ± 4.2	72.6 ± 3.8
Total wet weight	w_T	g	197.9 ± 21.3	197.5 ± 28.3	202.4 ± 18.7	232.6 ± 28.3	233.2 ±
Tail wet weight	w_A	g	61.7 ± 8.4	60.5 ± 10.2	62.3 ± 5.9	85.8 ± 10.3	87.9 ± 9.9
Tail Index	TI	% w_T	31.1 ± 1.9	30.7 ± 3.6	30.8 ± 1.7	36.9 ± 1.9	37.7 ± 2.7
Hepatopancreas wet	w_H	g	8.8 ± 1.7	10.6 ± 2.0	10.3 ± 2.3	11.7 ± 1.9	11.9 ± 1.7
Hepatosomatic Index	HSI	% w_T	4.5 ± 0.9	5.5 ± 1.2	5.1 ± 1.1	5.1 ± 1.0	5.2 ± 0.9
Moult Stage	numeric		1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.6 ± 0.5	1.7 ± 0.4
Females							
n			25	25	25	25	25
Carapace length	CL	mm	72.8 ± 4.0	73.9 ± 4.1	72.0 ± 4.1	72.4 ± 3.5	72.0 ± 4.1
Total wet weight	w_T	g	230.8 ± 33.3	233.4 ± 27.4	213.0 ± 30.2	216.2 ± 36.9	213.0 ±
Tail wet weight	w_A	g	86.6 ± 14.1	85.8 ± 10.3	76.1 ± 9.1	78.3 ± 11.7	76.1 ± 9.1
Tail Index	TI	% w_T	37.4 ± 1.5	36.8 ± 2.4	35.9 ± 2.2	36.8 ± 6.0	35.9 ± 2.2
Hepatopancreas wet	w_H	g	9.4 ± 1.3	10.8 ± 1.4	8.7 ± 1.9	8.7 ± 1.4	9.9 ± 1.9
Hepatosomatic Index	HSI	% w_T	4.1 ± 0.7	4.7 ± 0.7	4.1 ± 1.1	4.1 ± 0.9	4.8 ± 1.2
Gonad wet weight	w_O	g	3.9 ± 1.4	5.5 ± 2.2	5.8 ± 2.1	7.0 ± 3.1	8.6 ± 3.7
Gonadosomatic Index	GSI	% w_T	1.7 ± 0.6	2.4 ± 0.9	2.8 ± 1.1	3.4 ± 1.6	4.2 ± 2.2
Moult Stage	numeric		1.0 ± 0.0	1.1 ± 0.3	1.4 ± 0.6	1.6 ± 0.6	2.0 ± 0.6
Gonad stage	numeric		2.7 ± 0.5	3.0 ± 0.5	3.3 ± 0.6	3.6 ± 0.6	3.2 ± 0.9

Values are given as mean ± SD. n.d. = not determined. Abbreviations: CL = carapace length, w_T = total wet weight, w_A = tail wet weight, w_H = hepatopancreas wet weight, w_O = ovary wet weight, TI = Tail Index, HSI = Hepatosomatic Index, GSI = Gonadosomatic Index.

Ovary stages were classified according to Heydorn (1969) from stages 1 (immature) to 6 (spent) according to coloration and relative size (see Table 1). An increasing average numerical moult stage indicates progress towards moult.

Analysis of lipids was performed by the Council of Scientific and Industrial Research (CSIR) of South Africa as follows: After removal from the freezer, hepatopancreas weight (w_H) and gonad weight (w_O) were determined, the sample defrosted and homogenized using a Ultraturrax blender (IKA, Germany). Subsequently, fats and cholesterol were extracted by acid hydrolysis, adding pyrogallic acid to minimise oxidation, triundecanoin acid ($C_{11:0}$) and cholestane were added as internal standards (AOAC method 996.06, 2000). Fats and cholesterol were then extracted into ether and dried (AOAC method 996.06, 2000). Thereafter, fatty acids were methylated using dimethyl ether and identified and quantified by gas chromatography (AOAC method 996.06, 2000). Total fat was calculated as the sum of individual fatty acids expressed as triglyceride equivalents. Note: This is different to the determination of total lipid content in the previous chapters. From the dried extract, cholesterol was derivatised using HDMS, TMCS and dimethylformamide and subsequently subjected to gas chromatography for quantitation (AOAC 994.10, 2012).

Statistical differences of biological and biochemical parameters between moult stages and ovary maturation stages were analyzed by one-way ANOVA followed by the appropriate parametric- or nonparametric post-hoc test (given in figure and table captions). All statistics were calculated by using Sigma Plot version 14.0. The significance level chosen throughout was $p < 0.05$.

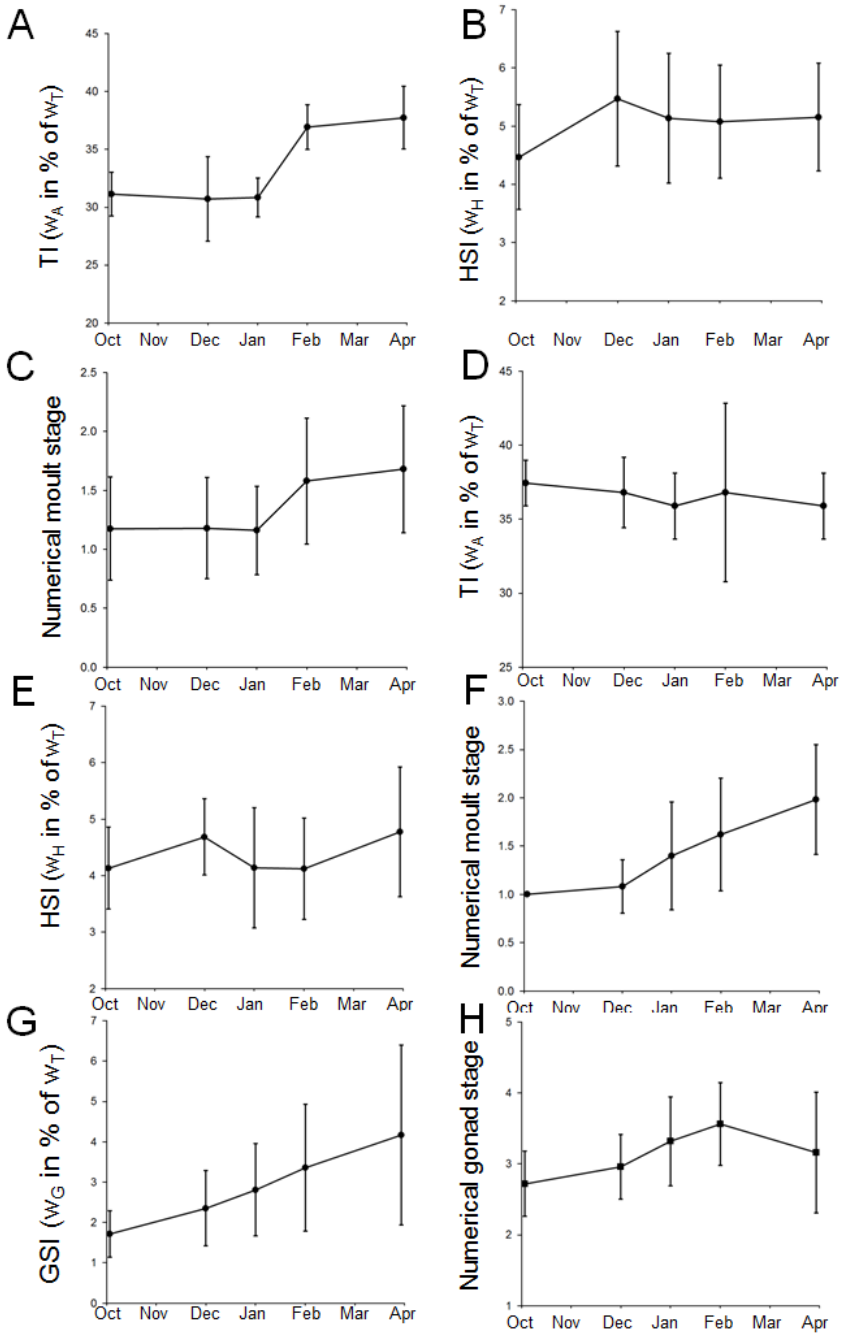

Figure 1. Progression of biological parameters of male and female *J. lalandii* caught during the period from October 2014 to April 2015. A) male Tail Index (TI), B) male Hepatosomatic Index (HSI), C) male numerical moult index, D) female TI, E) female HSI, F) female numerical moult index, G) female Gonatosomatic Index (GSI) and H) female numerical gonad index. Values are given as means ± S.D. n = 20 – 25. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

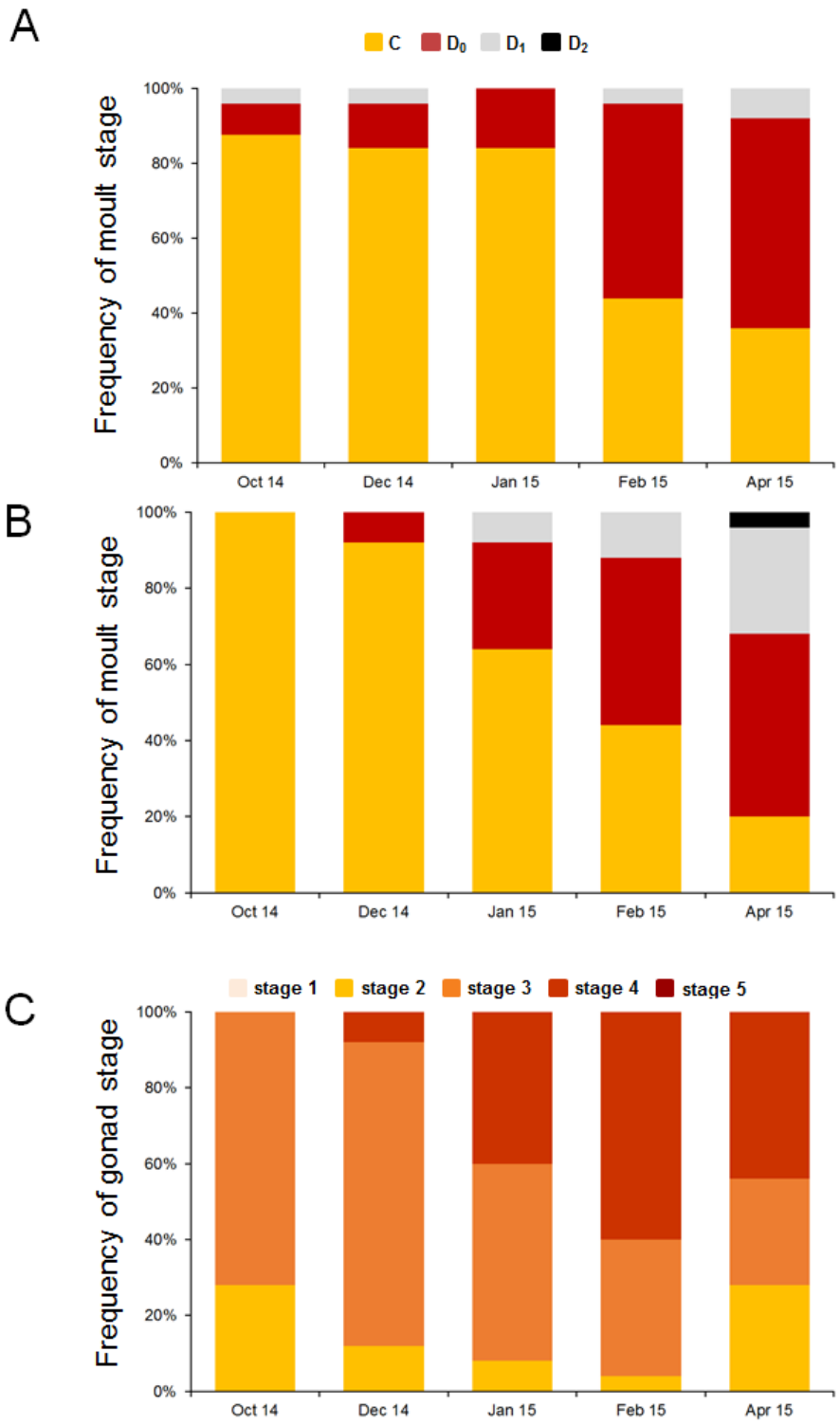

Figure 2. Relative frequency distribution of A) male and B) female moult stages and C) frequency of female gonad stages of *J. lalandii* caught during the period from October 2014 to April 2015. n = 20 – 25.

5.3 Results

Biological data

In males mean total wet weight varied from 197.5 to 233.2 g and CL from 70.8 to 73.6 mm whereas in females w_T varied from 213 to 233.4 g and CL from 72 to 73.9 mm (Table1). Mean wet tail (abdomen) weight (w_A) of males ranged from 60.5 g to 87.9 g and from 76.1 g to 86.6 g in females (Table 1). Average TI of males increased during the sampling period and ranged from around 31% of w_T in December to 38% in April (Table 1, Figure 1A). In females, TI ranged from around 36% to just over 37% with no clear trend evident (Table 1, Figure 1D). The mean hepatopancreas wet weight (w_H) of males ranged from 9 g in October to 12 g in April and from close to 9 g in January and February to about 11 g in December in females (Table1). In males, mean Hepatosomatic Index (HSI) ranged from 4.5% of w_T to 5.5% and from 4.1% to 4.8% in females. No clear trend in either male or female HSI was apparent (Table1, Figure 1B, 1E).

Moult cycle

The stage in the moult cycle was determined from all animals in the present study (see methods section comment). Moult stages C, D_0 and D_1 were detected in both males and females with stage D_2 only found in females (Figure 2A and B). The majority (85%) of males were in stage C in October but only 36% were in this moult stage in April. The D_0 stage in males first appeared in December (8%) and reached a maximum proportion of 56% in April. Only a few animals in stage D_1 (0-2 each month) were recorded during the study (Figure 2A). The numerical moult stage indicator in males progressed from a plateau of 1.2 from October to January to a maximum of 1.7 in April (Table 1, Figure 1C). The progress of the female moult cycle was similar to that of males. All females (100%) were in stage C in October and only 20% in April. Females in stage D_0 first appeared in December and increased to a maximum of 48% in April. Females in stage D_1 increased from 8% in January to 28% in April. One female in stage D_2 (4%) was caught in April (Figure 2B). The numerical moult indicator of females advanced from 1.0 in October to 2.0 in April (Table 1, Figure 1F).

Ovarian cycle

Both ovary wet weight ($_3$ 3.9 to 8.6g) and GSI (1.7 to 4.2%) increased substantially from October to April (Table 1, Figure 1G). Only ovary maturation stages 2 to 4 were found in the females sampled (Figure 2C). The proportion of animals in stage 2 declined from 28% in October to 4% in February and increased again to 28% in April (Figure 2C).

Table 2. Lipid composition of male and female hepatopancreas and female gonads from *J. lalandii* caught from October 2014 to April 2015.

		Oct 2014	Dec 2014	Jan 2015	Feb 2015	Apr 2015
Males						
n		20	25	25	25	25
Hepatopancreas						
Total fat	mg g^{-1} w_W	4.9 ± 2.7	8.0 ± 4.1	7.8 ± 2.7	9.4 ± 3.5	8.0 ± 3.5
Cholesterol	mg g^{-1} w_W	109 ± 30	109 ± 24	131 ± 31	123 ± 26	130 ± 26.4
Major fatty acids:	% of all FA					
$C_{14:0}$ (Myristic acid)		2.2 ± 0.4	2.5 ± 0.6	2.9 ± 0.5	3.1 ± 0.6	2.8 ± 0.8
$C_{16:0}$ (Palmitic acid)		15.3 ± 5.3	15.9 ± 1.1	15.0 ± 1.1	15.5 ± 1.1	16.1 ± 1.2
$C_{16:1(n-7)}$ (Palmitoleic acid)		6.5 ± 1.3	7.6 ± 1.2	7.8 ± 1.1	8.4 ± 1.3	6.6 ± 1.6
$C_{18:0}$ (Stearic acid)		8.0 ± 1.2	7.2 ± 1.0	6.8 ± 0.8	6.1 ± 0.7	7.1 ± 1.1
$C_{18:1}$ (Oleic acid)		20.7 ± 2.4	21.0 ± 1.9	21.2 ± 1.6	20.9 ± 1.7	19.2 ± 1.7
$C_{18:2(n-6)}$ (Linoleic acid)		1.1 ± 0.6	1.2 ± 0.5	1.1 ± 0.4	1.2 ± 0.4	1.2 ± 0.2
$C_{18:3(n-3)}$ (Linolenic acid)		4.3 ± 0.9	3.3 ± 1.3	3.2 ± 0.9	3.6 ± 0.7	3.9 ± 1.9
$C_{20:4(n-6)}$ (Arachidonic acid)		5.5 ± 1.3	5.6 ± 2.0	6.4 ± 1.9	5.7 ± 2.0	6.6 ± 1.7
$C_{20:5(n-3)}$ (Eicosapentaenoic acid)		18.6 ± 2.1	18.8 ± 1.7	19.0 ± 1.4	18.8 ± 1.5	17.2 ± 1.5
$C_{22:6(n-3)}$ (Docosahexaenoic acid)		17.7 ± 4.5	16.8 ± 1.3	16.7 ± 1.8	16.7 ± 2.2	19.3 ± 2.5
Σ SFA		25.5 ± 5.1	25.6 ± 1.6	24.6 ± 1.2	24.7 ± 1.3	26.0 ± 1.1
Σ MUFA		27.2 ± 3.2	28.7 ± 1.5	29.0 ± 1.6	29.3 ± 1.9	25.8 ± 1.4
Σ PUFA		47.3 ± 4.7	45.7 ± 1.4	46.4 ± 1.8	46.0 ± 2.1	48.2 ± 1.5
Females						
n		25	25	25	25	25
Hepatopancreas						
Total fat	mg g^{-1} w_W	5.4 ± 1.7	7.7 ± 4.0	7.5 ± 2.2	8.9 ± 2.9	7.2 ± 3.6
Cholesterol	mg g^{-1} w_W	102.0 ± 14.0	110.7 ± 22.8	126.7 ± 23.1	114.8 ± 30.2	122.5 ± 25.7
Major fatty acids:	% of all FA					
$C_{14:0}$		2.8 ± 0.3	2.7 ± 0.6	3.8 ± 0.5	3.8 ± 0.5	3.6 ± 0.8
$C_{16:0}$		17.7 ± 0.9	17.0 ± 0.8	18.2 ± 0.8	17.6 ± 0.7	18.5 ± 0.9
$C_{16:1(n-7)}$		10.3 ± 1.6	8.1 ± 1.2	10.3 ± 1.3	9.8 ± 1.2	9.2 ± 1.3
$C_{18:0}$		6.8 ± 0.9	7.7 ± 1.1	6.6 ± 0.5	6.1 ± 0.6	7.1 ± 0.8
$C_{18:1}$		16.1 ± 0.8	15.4 ± 0.7	16.5 ± 0.7	16.0 ± 0.6	16.7 ± 0.8
$C_{18:2(n-6)}$		1.4 ± 0.5	1.3 ± 0.6	1.6 ± 0.4	1.5 ± 0.4	1.5 ± 0.5
$C_{18:3(n-3)}$		3.7 ± 0.5	3.5 ± 1.3	3.3 ± 0.6	3.8 ± 0.7	3.8 ± 1.6
$C_{20:4(n-6)}$		5.3 ± 1.5	6.0 ± 2.1	6.3 ± 1.3	5.1 ± 1.0	6.3 ± 1.8
$C_{20:5(n-3)}$		18.5 ± 3.4	20.2 ± 2.3	18.7 ± 2.2	19.7 ± 2.6	18.0 ± 2.7
$C_{22:6(n-3)}$		17.5 ± 1.3	18.0 ± 1.3	14.8 ± 1.2	16.6 ± 1.6	15.2 ± 1.5
Σ SFA		27.3 ± 1.4	27.4 ± 1.2	28.6 ± 1.0	27.5 ± 1.0	29.1 ± 1.4
Σ MUFA		26.4 ± 2.2	23.6 ± 1.5	26.8 ± 1.7	25.8 ± 1.4	26.0 ± 1.5
Σ PUFA		46.3 ± 3.3	49.0 ± 2.2	44.6 ± 2.4	46.7 ± 1.9	44.9 ± 2.3
Ovaries						
Total fat	mg g^{-1} w_W	10.6 ± 2.3	10.3 ± 2.7	11.4 ± 1.0	11.2 ± 1.3	11.3 ± 3.8
Cholesterol	mg g^{-1} w_W	295.1 ± 62.0	269.7 ± 82.0	279.8 ± 40.6	351.6 ± 41.0	349.2 ± 42.2
Major fatty acids:	% of all FA					
$C_{14:0}$		1.9 ± 0.2	1.9 ± 0.4	2.1 ± 0.2	2.3 ± 0.3	2.6 ± 0.7
$C_{16:0}$		16.8 ± 0.7	17.1 ± 0.6	17.3 ± 0.9	17.3 ± 0.9	17.6 ± 0.7
$C_{16:1(n-7)}$		12.5 ± 1.5	11.3 ± 2.9	12.4 ± 2.1	11.3 ± 1.4	10.3 ± 1.2
$C_{18:0}$		6.2 ± 0.5	6.3 ± 1.1	5.6 ± 0.7	5.9 ± 0.4	6.4 ± 0.8
$C_{18:1}$		15.3 ± 0.6	15.6 ± 0.6	15.8 ± 18.7	15.8 ± 0.8	16.0 ± 0.7
$C_{18:2(n-6)}$		1.2 ± 0.5	0.9 ± 0.3	1.3 ± 0.4	1.2 ± 0.3	1.3 ± 0.4
$C_{18:3(n-3)}$		2.0 ± 0.3	2.6 ± 1.5	1.6 ± 0.2	1.8 ± 0.3	1.8 ± 1.0
$C_{20:4(n-6)}$		6.1 ± 1.1	5.6 ± 1.7	7.3 ± 1.4	5.7 ± 1.2	6.3 ± 2.0
$C_{20:5(n-3)}$		21.1 ± 2.2	20.6 ± 1.8	21.3 ± 2.1	22.3 ± 1.9	21.8 ± 3.1
$C_{22:6(n-3)}$		17.0 ± 1.7	17.9 ± 1.9	15.4 ± 1.9	16.3 ± 1.3	15.9 ± 1.6
Σ SFA		24.8 ± 0.8	25.3 ± 1.2	25.0 ± 1.0	25.5 ± 0.8	26.7 ± 1.5
Σ MUFA		27.7 ± 1.8	27.0 ± 3.2	28.2 ± 2.6	27.1 ± 1.8	26.4 ± 1.5
Σ PUFA		47.4 ± 2.5	47.7 ± 2.6	46.9 ± 3.4	47.4 ± 2.3	47.0 ± 2.4

Values are given as mean ± SD. Abbreviations: Σ SFA = total saturated fatty acids, Σ MUFA = total monounsaturated fatty acids, Σ PUFA = total polyunsaturated fatty acids, FA = fatty acids, w_W = wet weight.

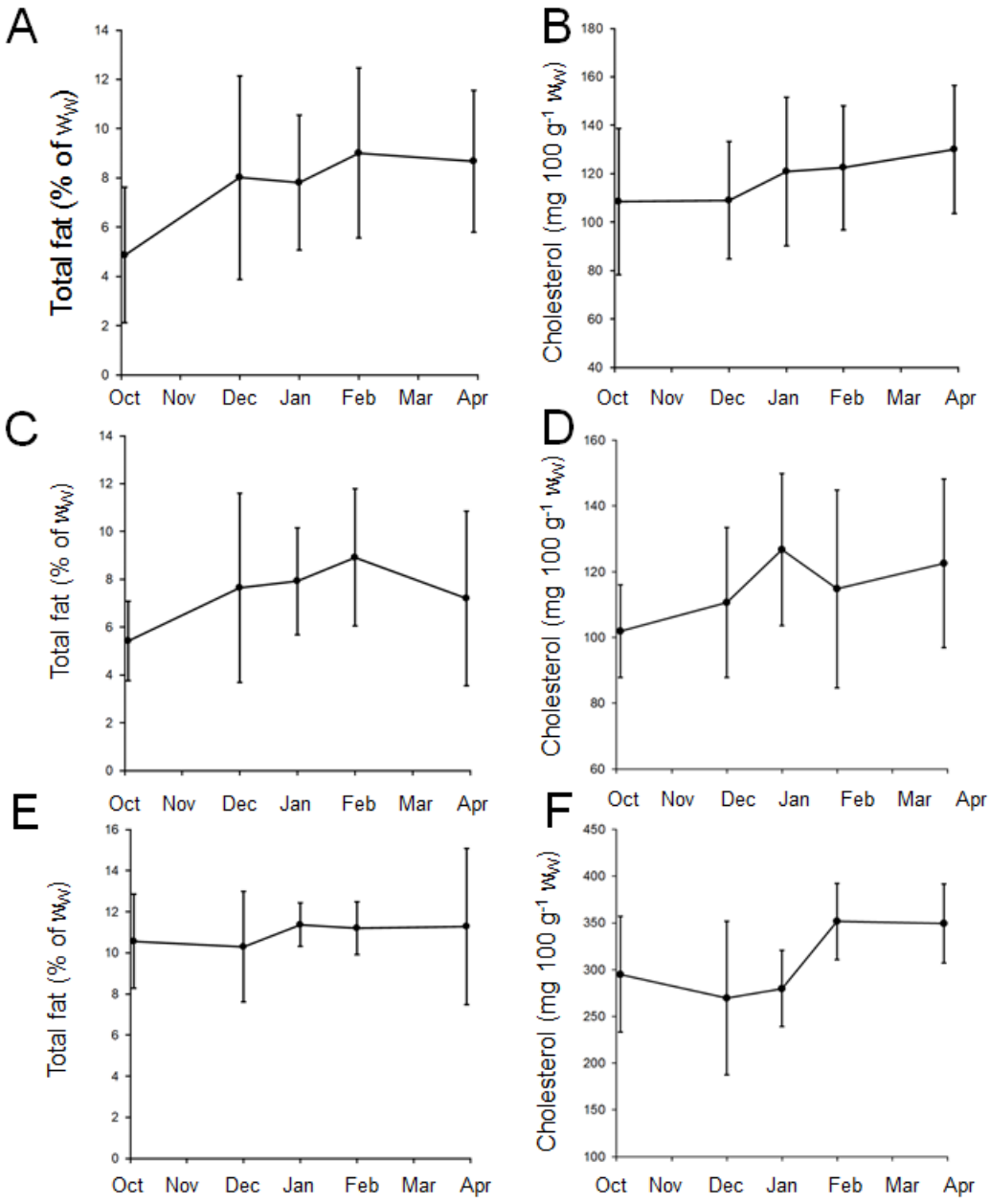

Figure 3. Course of concentrations of A) male hepatopancreas total fat, B) male hepatopancreas cholesterol, C) female hepatopancreas total fat, D) female hepatopancreas cholesterol, E) female gonad total fat and F) female gonad cholesterol of *J. lalandii* caught during the period from October 2014 to April 2015. Values are means ± S.D., n = 20 – 25. Abbreviation: w_W = wet weight.

Overall, most females were in stage 3, with the proportion of this stage declining from 72 and 80% in October and December to 28% in April. In December, a few females (8%) in stage 4 appeared, from which their proportion increased to 60% in February, followed by a decline to 44% in April. The numerical indicator of gonad stage increased from 2.7 in October to 3.6 in February after which it declined to 3.2 in April (Table 1, Figure 2H).

Organ lipids

Total fat and cholesterol contents as well as the fatty acid profiles (of total fatty acids) were analysed for male and female hepatopancreas and female gonads (ovaries) throughout the sampling period (Tab. 2).

Male hepatopancreas: Total fat content almost doubled from October to February and then declined by 16% in April (Table 2, Figure 3A). Cholesterol content rose by 19% from October to April (Table 2, Figure 3B). Principal fatty acids included $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$, which comprised approximately 70% of the 10 major FA. Fatty acid composition was relatively unchanged during the sampling period (Table 2). The proportion of polyunsaturated fatty acids (PUFA) was high: close to 48%, followed by ~25% saturated fatty acids (SFA) and ~29% monounsaturated fatty acids (MUFA). These proportions remained relatively stable.

Female hepatopancreas: Total fat content increased by more than 60% from October to February after which it declined by 23% until April (Table 2, Figure 3C). Cholesterol content rose by approximately 20% from October to April (Table 2, Figure 3D). Principal fatty acids included $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$, which comprised ~70% of the ten major FA. Fatty acid composition was relatively unchanged during the sampling period (Table 2). The proportion of PUFA was high: ~45%, followed by ~28% SFA and more than 26% MUFA. These proportions remained relatively stable. There is a higher proportion of SFA and a lower of PUFA than in the male hepatopancreas.

Female gonads: Total fat content increased marginally by 7% from October to April (Table 2, Figure 3E). Cholesterol content rose by 19% from October to February and remained at this level till April (Table 2, Figure 3F). Principal fatty acids included $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$, which comprised ~70% of the ten major FA. Fatty acid composition was relatively unchanged during the sampling period (Table 2). The share of PUFA was high at ~47%, followed by ~26% SFA and ~27% MUFA. These proportions remained relatively stable.

Biological and biochemical parameters during the moult cycle

Body parameters of male and female lobsters were analysed and arranged in relation to moult stage. Moult stages present were C to D_2 (see above).

Males: TI increased slightly by 10% from stage C to D_1 (Table 3, Figure 4A), whereas HSI declined slightly by 8% (Table 3, Figure 4B). Hepatopancreas fat content increased steadily by 35% from stage C to stage D_1 (Table 3, Figure 4C) and cholesterol increased concomitantly by 16% (Table 3, Figure 4D).

Table 3. Lipid composition of male and female hepatopancreas and female gonads of *J. lalandii* caught from October 2014 to April 2015 analysed per moult stage.

		Moult stage			
		C	D_0	D_1	D_2
Males					
n		79	36	5	
TI		32.5 ± 3.5	35.6 ± 4.4*	35.8 ± 3.2	
HSI		5.1 ± 1.1	5.1 ± 0.8	4.7 ± 1.6	
Hepatopancreas					
Total fat	mg g^{-1} w_W	7.1 ± 3.6	8.7 ± 3.5*	9.7 ± 3.2	
Cholesterol	mg g^{-1} w_W	115.0 ± 27.6	126.2 ± 27.2	132.8 ± 35.6	
Major fatty acids:	% of all FA				
$C_{14:0}$		2.6 ± 0.7	2.9 ± 0.6*	3.0 ± 0.6	
$C_{16:0}$		15.4 ± 2.8	15.9 ± 1.2	15.4 ± 1.2	
$C_{16:1(n-7)}$		7.2 ± 1.6	7.7 ± 1.1	7.7 ± 2.0	
$C_{18:0}$		7.2 ± 1.6	6.7 ± 0.8*	6.8 ± 1.2	
$C_{18:1}$		21.1 ± 1.9	19.8 ± 1.9*	19.2 ± 1.3	
$C_{18:2(n-6)}$		1.1 ± 0.5	1.2 ± 0.4	1.5 ± 0.4	
$C_{18:3(n-3)}$		3.5 ± 1.2	3.8 ± 1.1	4.5 ± 2.8	
$C_{20:4(n-6)}$		5.9 ± 1.9	6.0 ± 1.7	6.7 ± 2.8	
$C_{20:5(n-3)}$		18.8 ± 1.7	17.8 ± 1.7*	17.2 ± 1.2*	
$C_{22:6(n-3)}$		17.2 ± 3.0	18.0 ± 2.1	18.0 ± 2.5	
Σ SFA		25.1 ± 2.8	25.6 ± 1.5	25.2 ± 1.3	
Σ MUFA		28.3 ± 2.5	27.6 ± 2.1	26.9 ± 1.7	
Σ PUFA		46.6 ± 2.9	46.8 ± 1.7	47.9 ± 2.6	
Females					
n		79	32	13	1
TI		36.9 ± 1.9	35.9 ± 5.7*	36.2 ± 1.5	37.1
HSI		4.3 ± 0.9	4.2 ± 0.9	5.0 ± 1.3*	6.2
GSI		2.6 ± 1.6	3.0 ± 1.4	4.0 ± 1.9*	2.8
Gonad stage		3.0 ± 0.6	3.5 ± 0.6*	3.2 ± 0.8	2.0
Hepatopancreas					
Total fat	mg g^{-1} w_W	6.8 ± 3.4	8.4 ± 3.1*	8.3 ± 3.1	7.3
Cholesterol	mg g^{-1} w_W	110.9 ± 24.1	120.8 ± 25.3	129.0 ± 23.2*	141.0
Major fatty acids:	% of total FA				
$C_{14:0}$		3.1 ± 0.7	3.8 ± 0.6*	3.7 ± 0.6*	3.7
$C_{16:0}$		18.1 ± 1.2	18.4 ± 0.8*	18.9 ± 0.8*	19.3
$C_{16:1(n-7)}$		9.5 ± 1.7	9.5 ± 1.3	9.7 ± 0.8	9.6
$C_{18:0}$		7.0 ± 1.0	6.7 ± 0.7	6.4 ± 0.9	7.5
$C_{18:1}$		16.0 ± 0.8	16.4 ± 0.8*	16.9 ± 0.7*	16.1
$C_{18:2(n-6)}$		1.4 ± 0.5	1.6 ± 0.3	1.4 ± 0.6	2.0
$C_{18:3(n-3)}$		3.5 ± 1.1	3.7 ± 0.9	3.5 ± 1.1	4.7
$C_{20:4(n-6)}$		5.7 ± 1.8	6.2 ± 1.4	5.5 ± 1.2	5.9
$C_{20:5(n-3)}$		19.3 ± 2.9	18.6 ± 2.6	18.8 ± 2.3	15.7
$C_{22:6(n-3)}$		17.0 ± 1.8	15.5 ± 1.7*	15.4 ± 1.2*	15.5
Σ SFA		27.7 ± 1.3	28.5 ± 1.5*	28.8 ± 1.2*	30.5
Σ MUFA		25.5 ± 2.2	25.9 ± 1.6	26.6 ± 1.2	25.7
Σ PUFA		46.9 ± 2.9	45.6 ± 2.7	44.6 ± 1.9*	43.8
Ovaries					
Total fat	mg g^{-1} w_W	10.5 ± 2.6	11.4 ± 2.1*	12.3 ± 1.4*	12.3
Cholesterol	mg g^{-1} w_W	290.4 ± 70.0	334.9 ± 42.0*	352.6 ± 46.7*	348.0
Major fatty acids:	% of total FA				
$C_{14:0}$		2.0 ± 0.4	2.3 ± 0.5*	2.4 ± 0.8	2.4
$C_{16:0}$		17.1 ± 0.8	17.3 ± 0.5	17.9 ± 0.8*	18.5
$C_{16:1(n-7)}$		12.1 ± 1.9	11.0 ± 1.6*	11.3 ± 1.0	12.1
$C_{18:0}$		6.0 ± 0.6	6.0 ± 0.8	6.2 ± 0.5	6.1
$C_{18:1}$		15.6 ± 0.8	15.8 ± 0.5	16.3 ± 0.7*	16.3
$C_{18:2(n-6)}$		1.1 ± 0.4	1.3 ± 0.3*	1.2 ± 0.3	1.3
$C_{18:3(n-3)}$		1.9 ± 0.4	1.7 ± 0.7*	1.8 ± 0.4	1.1
$C_{20:4(n-6)}$		6.1 ± 1.7	6.6 ± 1.6	5.7 ± 1.5	7.0
$C_{20:5(n-3)}$		21.4 ± 2.2	21.9 ± 2.1	21.5 ± 2.5	20.3
$C_{22:6(n-3)}$		16.7 ± 1.8	15.9 ± 1.9	15.8 ± 0.8*	14.8
Σ SFA		25.1 ± 1.0	25.7 ± 1.0*	26.5 ± 1.7*	27.0
Σ MUFA		27.7 ± 2.2	26.8 ± 1.9	27.5 ± 1.3	28.3
Σ PUFA		47.2 ± 2.7	47.5 ± 2.2	46.0 ± 2.4	44.7

Values are given as mean ± SD. Abbreviations: Σ SFA total saturated fatty acids, Σ MUFA total monounsaturated fatty acids, Σ PUFA total polyunsaturated fatty acids, FA fatty acids, w_W = wet weight. * Significant difference ($p < 0.05$) to intermoult (stage C) (one-way ANOVA followed by Dunn Analysis). Note: stage D_2 not included in analysis.

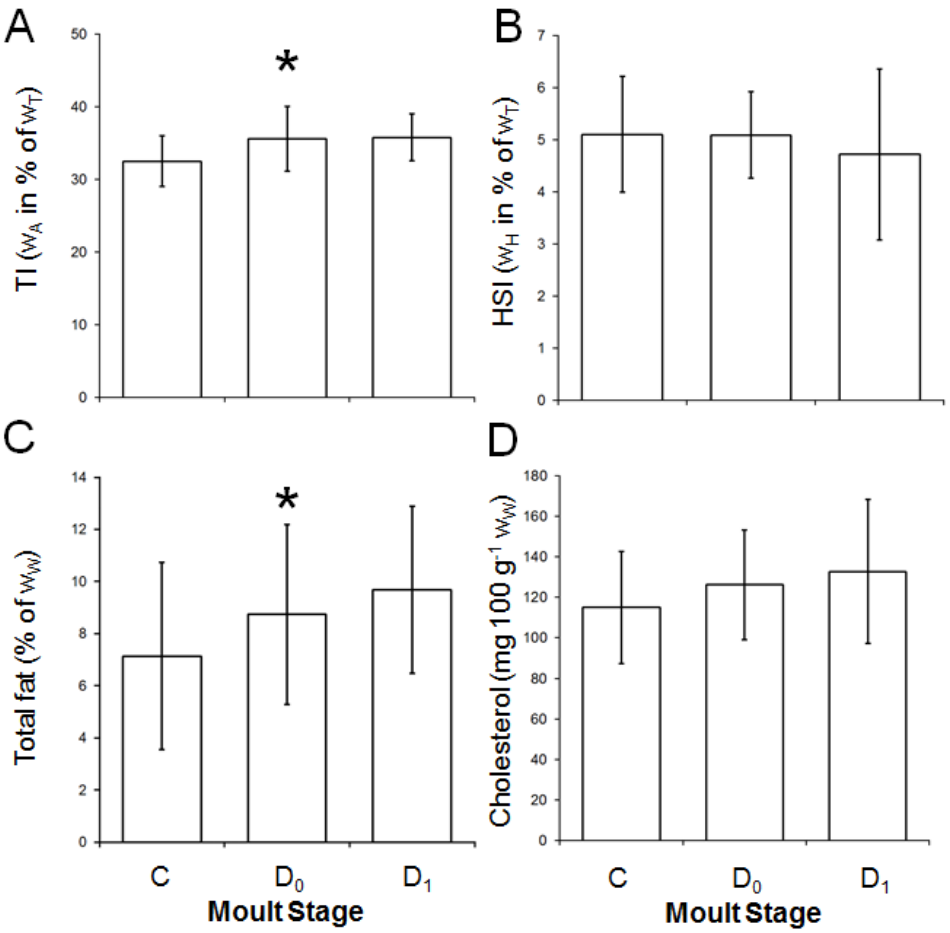

Figure 4. Variation in biological and biochemical parameters of male *J. lalandii* during the moult cycle. Data were analysed per moult stage: A) TI, B) HSI, C) total fat content and D) cholesterol content. Values are given as means ± S.D., for n per stage see Table 3. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_W = wet weight. *Significant difference ($p < 0.05$) to intermoult (stage C) (one-way ANOVA followed by Dunn Analysis).

In each moult stage found, the dominant FA were $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$ with a combined concentration of approximately 72%. Whereas $C_{18:3(n-3)}$ increased from a very low base (~3.5%), $C_{22:6(n-3)}$ declined by 11% (Table 3). The proportion of PUFA was highest at ~48%, followed by ~25% SFA and ~28% of MUFA. These proportions remained relatively stable.

Females: Only one animal was found in stage D_2. Data from this lobster is reported in Table 3 but was omitted in the statistical analysis. TI did not change from stage C to D_1 (Table 3, Figure 5A), whereas HSI increased from stage D_0 to D_1 by 19% (Table 3, Figure 5B). GSI increased throughout the observed moult cycle by 54% (Table 3, Figure 5C), whereas there was only a slight upward trend in the numerical gonad stage indicator (Table 3, Figure 5D). Hepatopancreas fat content increased steadily by 23% from stage C to stage D_1 (Table 3, Figure 5A) and cholesterol increased concomitantly by 16% (Table 3, Figure 5B).

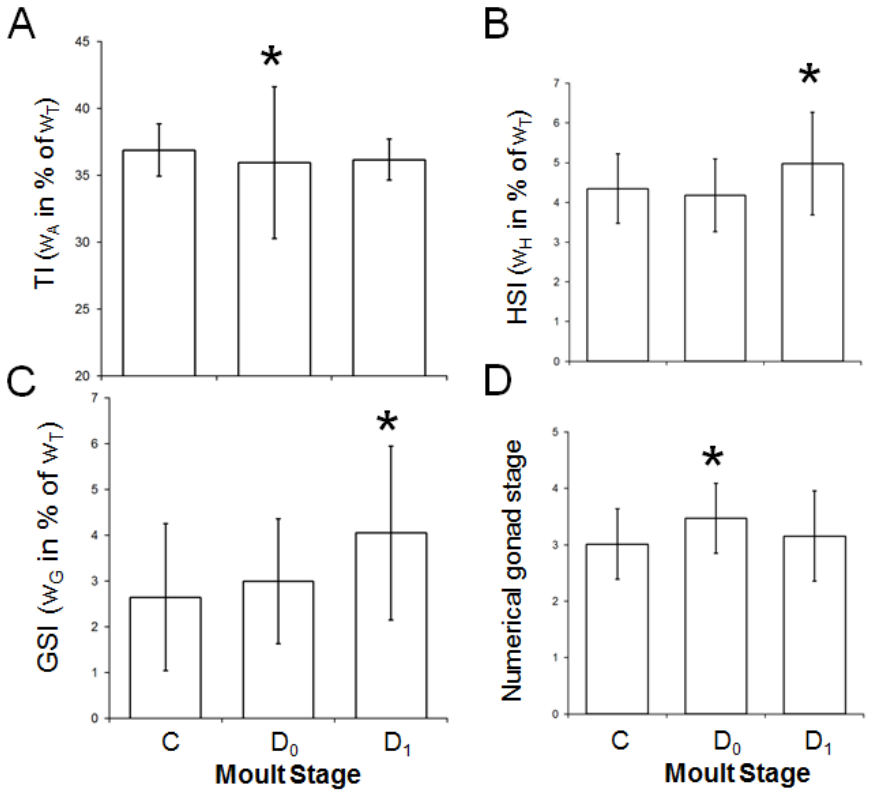

Figure 5. Variation in biological parameters of female *J. lalandii* during the moult cycle. Data were analysed per moult stage: A) TI, B) HSI, C) GSI and D) numerical gonad index. Values are given as means ± S.D., for n per stage see Table 3. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_O = ovary wet weight, w_W = wet weight. *Significant difference ($p < 0.05$) to intermoult (stage C) (one-way ANOVA followed by Dunn Analysis).

In each moult stage found, the dominant FA were $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$ with a combined concentration of more than 69%. There was a slight increase of $C_{16:0}$ and a concomitant decline of $C_{18:0}$ and $C_{22:6(n-3)}$ (Table 3). The proportion of PUFA was highest at ~45%, followed by ~29% SFA and >26% MUFA. The proportion of SFA increased slightly whereas that of PUFA declined concomitantly.

Ovary fat content increased steadily by 17% from stage C to stage D_1 (Table 3, Figure 6A) whilst cholesterol increased concomitantly by 13% (Table 3, Figure 6B). In each moult stage found, the five dominant FA were $C_{16:0}$, $C_{18:1}$, $C_{16:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$ with a combined proportion of >80%. A slight increase of $C_{16:0}$ occurred whereas $C_{22:6(n-3)}$ declined concomitantly (Table 3). The proportion of PUFA was high at ~47%, followed by ~26% SFA and ~27% MUFA. There was a slight increase of SFA and a concomitant decrease of PUFA.

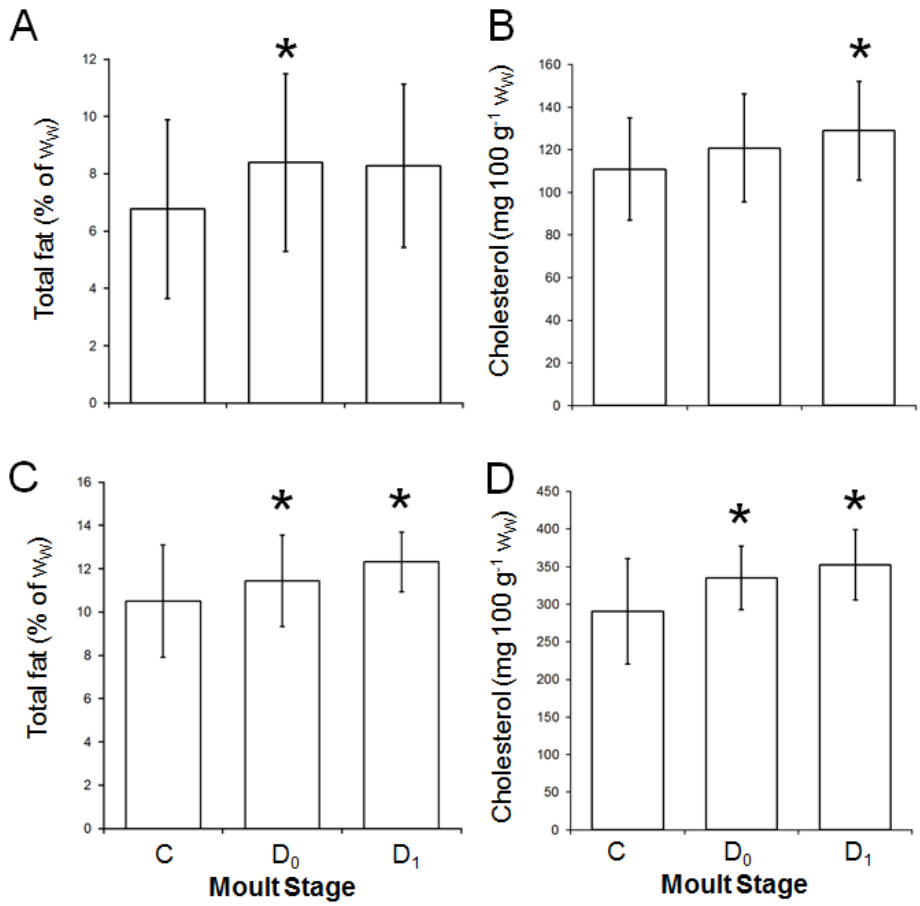

Figure 6. Variation in biochemical parameters of female *J. lalandii* during the moult cycle. Data were analysed per moult stage: Content of A) hepatopancreas total fat, B) hepatopancreas cholesterol, C) gonad total fat and D) gonad cholesterol. Values are given as means ± S.D., for n per stage see Table 3. Abbreviations: w_W = wet weight, w_D = dry weight. *Significant difference ($p < 0.05$) to intermoult (stage C) (one-way ANOVA followed by Dunn Analysis).

Biological and biochemical parameters during progression of ovary maturation

Parameters of female lobsters were analysed and arranged in relation to ovary maturation stages. Gonad stages present were 2 to 4 (see above).

TI decreased slightly by 5% from stage 2 to 4 (Table 4, Figure 7A), whereas HSI declined by 9% from stage 2 to stages 3 and 4 (Table 4, Figure 7B). GSI, however, increased substantially by 64% from stage 2 to 4 (Table 4, Figure 7C) and the moult stage indicator from 1.3 in stages 2 and 3 to 1.6 in stage 4 (Table 4, Figure 7D). Hepatopancreas fat content increased by ~10% from stages 2 and 3 to stage 4 (Table 4, Figure 8A) whereas cholesterol remained steady within a narrow range (Table 4, Figure 8B). The dominant FA in each gonad stage were $C_{16:0}$, $C_{18:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$ which accounted for close to 70% of all FA (Table 4). $C_{14:0}$ rose by 19% from a low base of 3.2% whereas $C_{18:2(n-6)}$ increased by 26% from a very low base from stage 2 to stage 4 (Table 4).

Table 4. Lipid composition of female hepatopancreas and gonads of *J. lalandii* caught from October 2014 to April 2015 analysed per gonad stage.

		Gonad stage		
		2	3	4
n		20	67	38
Indices				
TI	% of w_T	37.0 ± 2.1	37.2 ± 3.8	35.1 ± 2.4*
HSI	% of w_T	4.5 ± 0.8	4.5 ± 0.9	4.1 ± 0.9
GSI	% of w_T	2.2 ± 0.7	2.7 ± 1.3	3.6 ± 2.2*
Moult stage	numerical	1.3 ± 0.6	1.3 ± 0.5	1.6 ± 0.6*
Hepatopancreas				
Total fat	mg g^{-1} w_W	7.2 ± 4.1	7.0 ± 3.1	7.9 ± 2.8
Cholesterol	mg g^{-1} w_W	117.2 ± 24.8	112.3 ± 23.6	119.7 ± 27.7
Major fatty acids:	% of total FA			
$C_{14:0}$		3.2 ± 0.8	3.2 ± 0.8	3.7 ± 0.5*
$C_{16:0}$		18.0 ± 1.1	17.7 ± 1.0	17.9 ± 0.7
$C_{16:1(n-7)}$		9.9 ± 1.6	9.5 ± 1.6	9.4 ± 1.3
$C_{18:0}$		6.9 ± 0.9	6.9 ± 1.0	6.7 ± 0.9
$C_{18:1}$		16.3 ± 1.0	16.1 ± 0.9	16.2 ± 0.6
$C_{18:2(n-6)}$		1.3 ± 0.5	1.4 ± 0.5	1.6 ± 0.3
$C_{18:3(n-3)}$		3.7 ± 1.2	3.5 ± 1.1	3.8 ± 0.9
$C_{20:4(n-6)}$		5.7 ± 2.1	5.7 ± 1.7	6.0 ± 1.4
$C_{20:5(n-3)}$		18.3 ± 2.6	19.3 ± 3.0	18.9 ± 2.3
$C_{22:6(n-3)}$		16.7 ± 1.9	16.7 ± 2.0	15.8 ± 1.5
Σ SFA		28.1 ± 1.8	27.8 ± 1.5	28.2 ± 1.0
Σ MUFA		26.3 ± 2.0	25.5 ± 2.3	25.7 ± 1.5
Σ PUFA		45.6 ± 3.2	46.6 ± 3.2	46.1 ± 2.0
Ovaries				
Total fat	mg g^{-1} w_W	10.9 ± 3.6	10.9 ± 1.7	11.6 ± 1.9
Cholesterol	mg g^{-1} w_W	293.1 ± 60.0	310.5 ± 49.9	338.5 ± 48.3*
Major fatty acids:	% of total FA			
$C_{14:0}$		2.2 ± 0.7	2.0 ± 0.4	2.3 ± 0.4
$C_{16:0}$		17.5 ± 1.0	17.2 ± 0.7	17.3 ± 0.8
$C_{16:1(n-7)}$		11.6 ± 2.1	12.0 ± 1.7	11.5 ± 1.9
$C_{18:0}$		6.1 ± 0.9	6.0 ± 0.6	5.9 ± 0.4
$C_{18:1}$		15.9 ± 0.9	15.6 ± 0.6	15.7 ± 0.7
$C_{18:2(n-6)}$		1.4 ± 0.5	1.1 ± 0.4	1.2 ± 0.3
$C_{18:3(n-3)}$		1.8 ± 0.6	1.9 ± 0.6	1.7 ± 0.3
$C_{20:4(n-6)}$		5.7 ± 2.0	6.2 ± 1.5	6.5 ± 1.6
$C_{20:5(n-3)}$		20.8 ± 2.8	21.4 ± 2.0	22.1 ± 2.2
$C_{22:6(n-3)}$		16.8 ± 2.1	16.6 ± 1.7	15.8 ± 1.7
Σ SFA		25.9 ± 2.1	25.2 ± 1.0	25.5 ± 0.8
Σ MUFA		27.6 ± 2.3	27.7 ± 2.0	27.2 ± 2.2
Σ PUFA		46.6 ± 3.2	47.2 ± 2.4	47.3 ± 2.5

Values are given as mean ± SD. Abbreviations: Σ SFA total saturated fatty acids, Σ MUFA monounsaturated fatty acids, Σ PUFA polyunsaturated fatty acids, FA fatty acids, w_W = wet weight. *Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Dunn Analysis).

The remaining FA remained relatively unchanged. The proportion of PUFA was highest at ~46%, followed by ~28% SFA and >25% of MUFA. These proportions remained relatively stable (Table 4).

Ovary fat content increased slightly by 6% from stages 2 and 3 to stage 4 (Table 4, Figure 8C) whereas cholesterol rose by more than 15% from stage 2 to 4 (Table 4, Figure 8B).

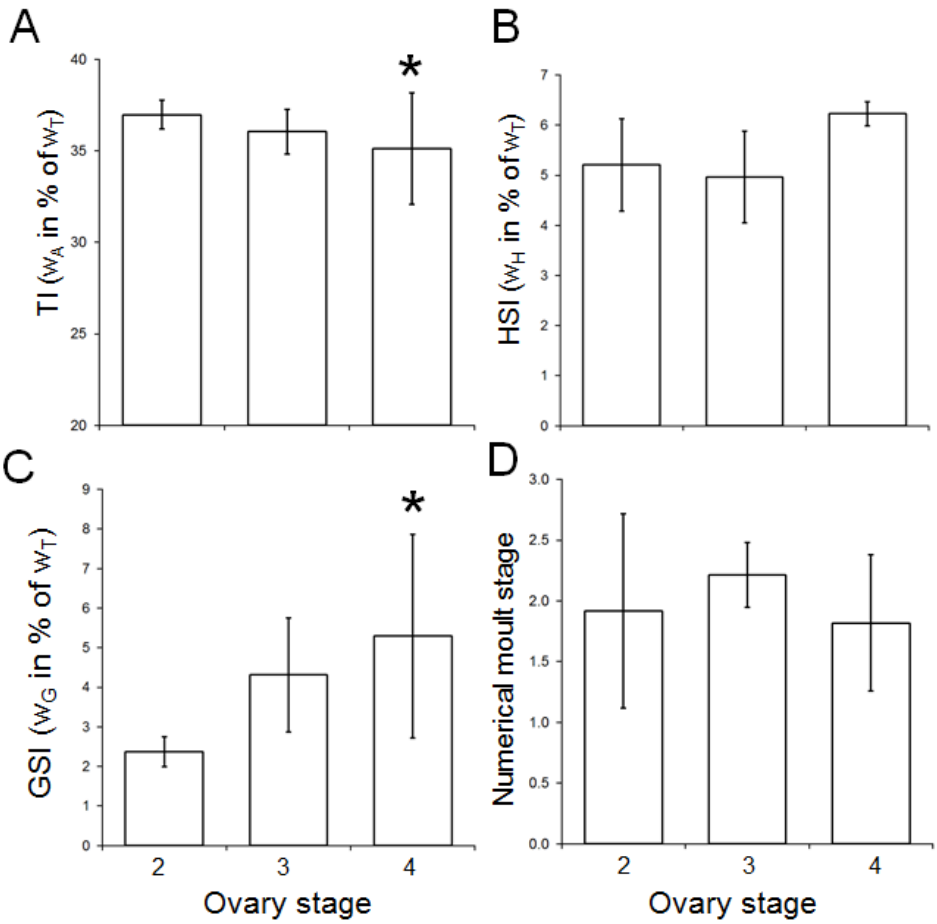

Figure 7. Variation in biological parameters of female *J. lalandii* during gonad maturation. Data were analysed per gonad (ovary) stage: A) TI, B) HSI, C) GSI and D) numerical moult index. Values are given as means ± S.D., for n per stage see Table 4. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_O = ovary wet weight, w_W = wet weight. *Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Dunn Analysis).

The dominant FA in each gonad stage were $C_{16:0}$, $C_{18:1}$, $C_{16:1}$, $C_{20:5(n-3)}$ and $C_{22:6(n-3)}$ with a proportion of ~70% of all FA (Table 4). $C_{20:4(n-6)}$ rose by 12% from a low base (5.7%) whereas $C_{20:5(n-3)}$ increased more than 6% from stage 2 to stage 4 (Table 4). Concomitantly, $C_{22:6(n-3)}$ declined by 6%. The remaining FA remained relatively unchanged. The proportion of PUFA was highest at ~47%, followed by >25% SFA and ~28% of MUFA. These proportions remained relatively stable during ovarian maturation (Table 4).

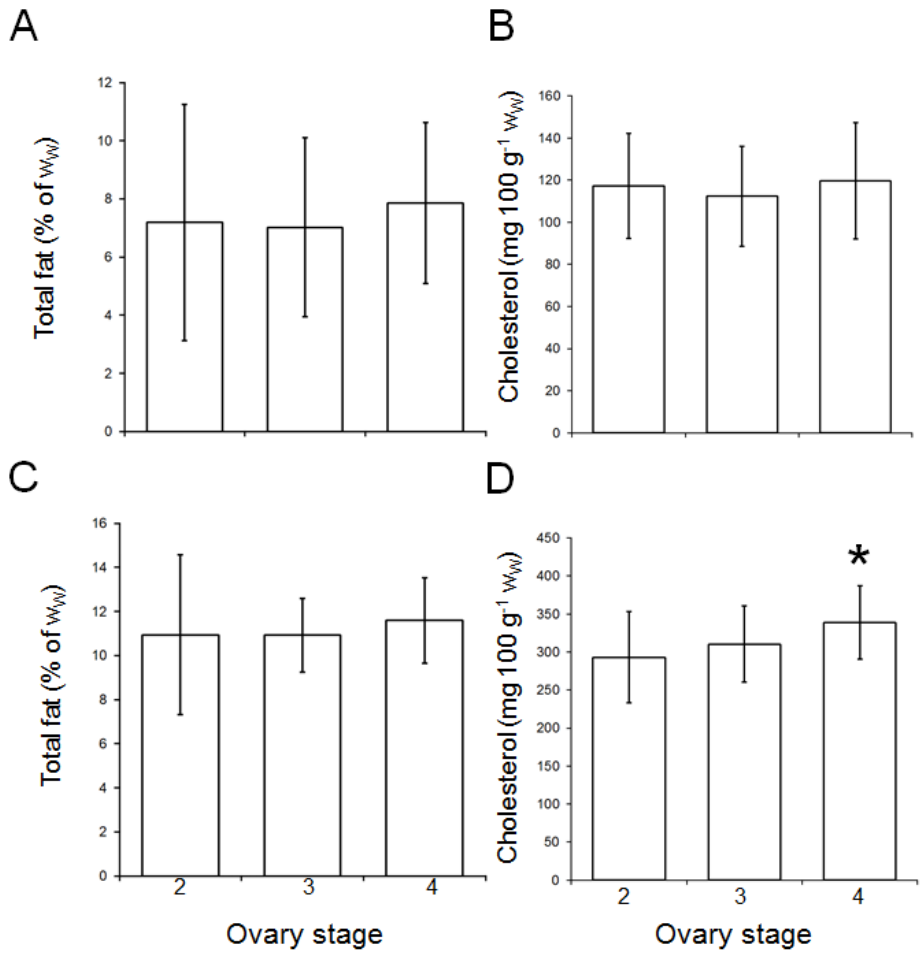

Figure 8. Variation in biochemical parameters of female *J. lalandii* during gonad maturation. Data were analysed per gonad (ovary) stage: Content of A) hepatopancreas total fat, B) hepatopancreas cholesterol, C) gonad total fat and D) gonad cholesterol. Values are given as means ± S.D., for n per stage see Table 4. Abbreviations: w_W = wet weight. *Significant difference ($p < 0.05$) to ovary stage 2 (one-way ANOVA followed by Dunn Analysis).

5.4 Discussion

It is well established that reserves are accumulated during the moult and reproductive cycles of male and female lobsters. It is however the amount of reserves accumulated that determines the relative success of somatic growth or reproductive output. In this chapter, the period of sampling (austral spring and summer or October - April) was selected to focus directly on the period of rapid reserve accumulation in the male hepatopancreas and female gonads to elucidate the more detailed aspects of lipid accumulation.

The results of this study are consistent with those from previous research (Cockcroft, 1997; van Rooy, 1998) and from Chapters 3 and 4: The period investigated displayed the expected build-up of reserve metabolites in male hepatopancreas and female ovaries. It also documented accumulation through the moult- (males and females) and ovary (females) cycles (Figure 1). In the reporting period, reserve accumulation in the male hepatopancreas is relatively fast from

October to December after which it reaches the peak plateau observed in Chapter 3. From February, preparation of moult began, seen by the appearance of more male lobsters in the pre-moult stage (Fig. 2A). This marks the beginning of reserve use for moult (growth) preparation (stage D_2, Charmantier-Daures and Vernet, 2004). Concomitant to the HSI increase, total fat content almost doubled until February whereas cholesterol content increased continuously by 20% until April. Intermoult is the longest phase during the moult cycle (Marco, 2012) during which reserve accumulation takes place over a prolonged period, starting at very low depot levels. Intermoult is therefore not homogenous, and levels of accumulated reserve metabolites change rapidly as the cycle progresses. The present sampling period covered a large portion of intermoult (biggest proportion of all moult stages), although most likely not the period of lowest reserve levels shortly after moult. Lipids, for example, accumulate mainly in sub-stage C_4 to early premoult and rapidly decline from D_2 (Charmantier-Daures and Vernet, 2004). At the beginning of sampling in October, HSI was still at a relatively low level (similar to levels recorded in Chapter 3), indicating that reserve accumulation had not yet commenced. However, total fat and cholesterol contents continue to increase as the moult cycle progresses. This slight lag of lipid peak relative to "reserve peak" (i.e. maximum hepatopancreas size, HSI) was observed previously (Chapter 3, Cockcroft, 1997). In contrast to males, female HSI does not show a trend, but total fat and cholesterol clearly accumulate. The ovary cycle is slightly ahead of the male moult cycle, explaining the lag in male HSI increase compared with the continuous increase in female GSI, numerical moult stage and frequency of more mature gonad stages until April. Fat and cholesterol from the hepatopancreas may be incorporated into egg- and embryo membranes during ovary maturation without accumulating in the hepatopancreas first (Harrison, 1990). Such a contribution of the hepatopancreas to ovary maturation, however, could not be estimated with the methods used here and needs to be investigated in future using biochemical methods and indicators.

There was no seasonal change in the FA profile of the male hepatopancreas. In the present study, the dominant saturated FA were $C_{16:0}$ at ~20% and $C_{18:0}$ at ~10% in *J. lalandii.* Similar results were found previously in *J. lalandii* (Lighthelm et al., 1953) and *J. edwardsii* (Nelson et al., 2005). Unsaturated FA were dominated by $C_{20:x}$ FA at ~30% (Table 2), similar to Lighthelm et al. (1953). In general, the proportion of mono- and unsaturated FA was high (~65%). The profile is similar in female hepatopancreas here (Table 2) where it is also more or less unchanged during the months of reserve build-up. This FA profile resembles that of female *J. edwardsii* hepatopancreas (Smith et al., 2004). Furthermore, the FA profile of *J. lalandii*

ovaries resembles that of male and female hepatopancreas (Table 2). Such similarity between the FA profiles of hepatopancreas and ovaries was also found in female *J. edwardsii* (Smith et al., 2004). However, hepatopancreas and muscle tissue differ more in FA profiles of (presumably male) *J. edwardsii* (Nelson et al., 2005). In *J. edwardsii*, starvation (Smith et al., 2004) and diet composition had an influence on the FA profile of female (Smith et al., 2004) and male hepatopancreas and abdominal muscle (Nelson, 2005). After prolonged feeding, fatty acid profile of the hepatopancreas resembled that of the diet (Smith et al., 2004). Interestingly, the FA profile in ovaries remained unchanged (Smith et al., 2004), pointing to a preferential reserve supply/use. This is possibly to ensure the presence of FA that are essential for crustaceans (Munian et al., 2020). The stable FA profiles in the present study indicate that food availability in the Hout Bay fishing area is unchanged and optimal for growth and reproduction of *J. lalandii* during the period of rapid reserve accumulation and that both growth and reproduction may depend on same food source.

During progression of the moult cycle, there is a slight decrease of HSI from C to D_1 in males. It is noteworthy, however, that only five animals made up the D_1 group which may have resulted in sampling bias. Total fat and cholesterol contents increased steadily and substantially from C to D_1. In females, GSI increases substantially whereas there is no trend in the HSI as the moult cycle progresses. This is an expression of the different proportions of reserves apportioned to growth in females (5%) when compared with males (99%). There is, however, a slight increase in total fat- and cholesterol contents during the transition towards pre-moult in the female hepatopancreas whereas in the ovaries, there is a clear and substantial upward trend of both by 17 and 21%, respectively, as ovary maturation progresses in parallel with the moult cycle.

The investigated period covers the early phase of ovary maturation after spawning, indicated by the exclusive presence of females in the ovarian maturation stages 2 to 4. Concomitantly, HSI fell by ~9%, whereas as expected, GSI increased from stage 2 to 4 by 64%. There was no change in lipid- and FA composition of the hepatopancreas, whereas there was a 7% fat increase and substantial cholesterol increase (16%) in the ovaries. Their FA composition remained unchanged during the moult cycle.

The abdominal muscle (tail) was not analysed in as much detail as hepatopancreas and ovaries here. However, analysis of its relative size revealed some interesting details: The male TI increased by 21% from October to April, which is more pronounced than previously found at

the same fishing area (Chapter 3) and may indicate a role in reserve accumulation. Moreover, a slight increase of TI from C to D_1 during the moult cycle was noted. In contrast to males, female TI slightly declined by 5% as the sampling period progressed. This, in turn, may indicate the use of reserves in the abdominal muscle for ovary maturation, i.e. another indication of a possible storage function. In addition, when analysed per ovarian maturation stage, female TI dropped by ~6% from stage 3 to 4. This also indicates a possible transfer of reserves from the abdominal muscle to the ovaries, possibly making use of the tail's high sterol and phospholipid content (Munian et al., 2021). Compared with the hepatopancreas (~5% of body weight), the abdominal muscle is large (~34% with exoskeleton). This larger size may, together with its higher sterol content, compensate for the tail's lower total lipid levels that are available for biological processes. These results warrant future biochemical research on the contribution of the abdominal muscle in ovary maturation and other processes.

For analysis of reserve partitioning between growth and reproduction, available depot fats per individual were compared for males and females, considering their different reserve prioritisation (see above). This comparison is possible, because males and females in the present study were of similar weight (Table 1). In males, the hepatopancreas is the only metabolic depot to fuel growth, whereas females have two - hepatopancreas and ovaries (see Chapter 2). The latter build up metabolic reserves as a supply for eggs/embryos and thus reproduction. This analysis revealed that, at any time point of the project period, females had substantially (42 – 120%) more fat available than males (Figure 9). The difference was persistently 0.5 – 0.6 g, except for April when moult preparation in males may already have commenced. There are several possible explanations for this difference: 1) females have a lower metabolic rate, allowing them to store a larger share of the ingested food, 2) females feed on food richer in lipids than males or, 3) females have a higher food consumption rate than males. However, there are no indications in palinurids that 1) is true: An expression of metabolic rate is oxygen consumption (respiration). In many decapods, however, no differences in oxygen consumption between sexes were found (Cockcroft and Wooldridge, 1985; Villarreal, 1990; Carvalho and Phan, 1997). This is also true for the few studies that investigated respiration of mature palinurids (*J. edwardsii*) from both genders (Waldron, 1991; Crear and Forteath, 2000). Results of the present study also make 2) unlikely: The hepatopancreas FA profile did not show any differences between males and females (Table 2). Such a difference in FA profile could have pointed at different diet compositions in males and females (Smith et al., 2004; Nelson, 2005).

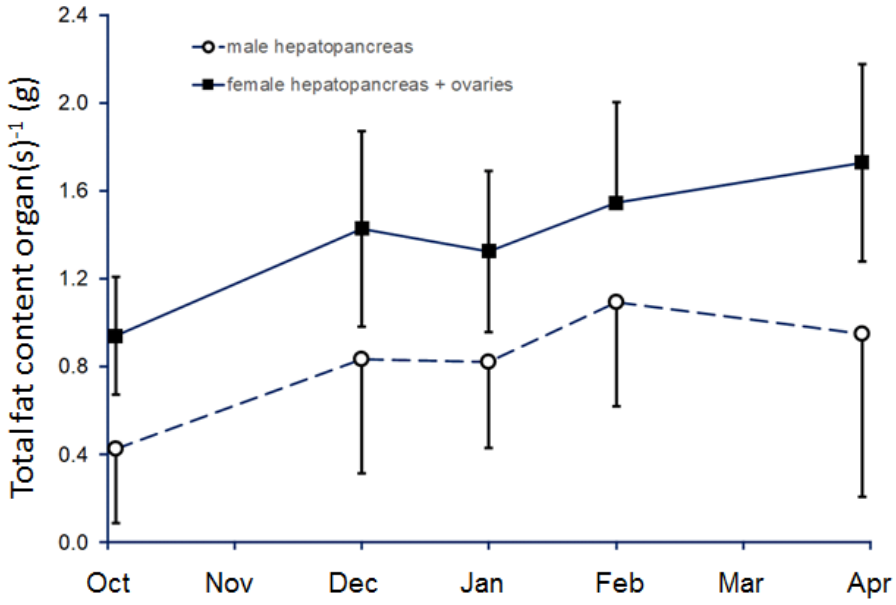

Figure 9. Amount of total fat in male hepatopancreas and the sum of both female hepatopancreas and ovaries of *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from October 2014 to April 2015. Values are means ± S.D., n = 20 – 25. Abbreviation: w_W = wet weight.

A higher female food consumption rate (3) is therefore the most likely explanation for the larger fat stores in females. If females can feed more than males in this area (Hout Bay), it means, in turn, that there is sufficient or surplus food for males to grow at an optimal rate. Regional (and inter-annual) growth differences are not well understood (Cockcroft, 1997) but are assumed to result from variations in food availability and composition (see Chapter 3). In other areas, notably Olifantsbos, where the annual growth increment is consistently lower by more than 1 mm (Chapter 3), feeding conditions should therefore be insufficient to support optimal male growth. This should be reflected in a lower or no fat surplus in females. Detailed, comparative research in different areas is required in future to proof the latter assumption. If proven correct, the gender difference in total fat stores could be suitable for assessment of food availability in a specific area and, hence, growth potential for males.

In contrast to Chapters 3 and 4, where the focus was on the periods of peak reserve accumulation, the present Chapter analysed the steep slope of accumulation in male hepatopancreas and in ovaries. Whereas these results show clear quantitative upward trends in male hepatopancreas and female ovary parameters, reflecting reserve accumulation, there are no distinct changes in qualitative properties, such as FA profile in all investigated organs during this period. The latter could have served as an indicator for successful accumulation during

preparation for male moult and female reproduction. In crabs, for example, changes in lipid class- and FA composition of the ovary were revealed during maturation (Mourente et al., 1994). No such data are available for palinurids so far.

5.5 Conclusion

The present chapter confirmed the rapid, parallel reserve accumulation in male hepatopancreas and female ovaries. Whereas total fat- and cholesterol contents in male hepatopancreas and female ovaries steadily increased, FA composition was stable in male and female hepatopancreas as well as in the ovaries throughout moult- and ovary maturation cycles. These stable FA profiles indicate that food availability in the Hout Bay fishing area is unchanged and optimal for growth and reproduction of *J. lalandii* during the period of rapid reserve accumulation. Furthermore, at any time point, females had substantially more body fat available than males, most likely indicating that same-size females have a higher food consumption rate and that the area provides sufficient or surplus food for optimal male growth.

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Chapter 6

General Discussion

The West Coast rock lobster (WCRL) *Jasus lalandii* is a key ecological species (Barkai and Branch, 1988) and supports one of the most important fisheries in South Africa (DEFF, 2020). This fishery is managed according to an operational management procedure (OMP) that provides the framework for the aim of sustainability and rebuilding the stock (DEFF, 2020). To achieve this, an annual Total Allowable Catch (TAC) for male WCRL is set scientifically in addition to some elements of effort control (DEFF, 2020). Despite these efforts, the harvestable component is currently estimated at 1.8% of pristine levels (DEFF, 2020).

Inputs for the TAC are Catch per Unit Effort (CPUE), a fisheries-independent abundance index resulting from annual monitoring surveys (FIMS), growth data of the previous season as well as estimates of catches of recreational-, small-scale- and related fisheries and poachers (DEFF, 2020). However, all these data are entirely based on data from the past and do not necessarily reflect the current state of the resource. The present research was therefore initiated to identify additional, timelier, indicators to set the TAC for the next season. The focus was at the physiological condition of the lobsters.

An important input into stock assessment is the somatic growth rate of commercial size male lobsters from each fishing area (Johnston et al., 2012). The analysis per area/zone is necessary because, historically, growth rates differ between areas (Cockcroft and Goosen, 1995). The two areas chosen for this study, Olifantsbos and Hout Bay, represent a low-growth and high-growth area, respectively. Regional growth differences are assumed to be a result of differences in food availability and composition (Newman and Pollock, 1974; Melville-Smith et al., 1995). Growth rates also differ interannually, depending on environmental conditions - especially food availability (Cockcroft and Goosen, 1995).

In general, the present research has confirmed historic growth trends as well as differences between the two chosen areas (Chapter 3; Goosen and Cockcroft, 1995; Cockcroft, 1997). Annual growth increment in the Hout Bay area is still substantially higher than in the Olifantsbos area (Chapter 3). Inter-annual growth differences are also still present, with growth rates ranging from 2.2 to 3.4 mm in Hout Bay and 1.5 to 2.3 mm in Olifantsbos for the period from 2008 to 2014 (Chapter 3). These values are in the same range as reported by Cockcroft (1997) for the period of 1992 – 1996. Values from both studies are therefore substantially lower than those before 1987, the year the slow growth period started (DEFF, 2020), indicating that

the factors for this slow growth generally persist. They were attributed to large-scale environmental changes that changed primary production in the Benguela system, which, in turn, reduced production and biomass of the preferred prey item of the WCRL - ribbed mussels (Shannon et al., 1992; Augustyn et al., 2018). From the conversion of Heydorn (1969b) it can be estimated that a difference in growth increment of 1 mm of a (standardized) 200 g lobster will result in a body weight differential of almost 3 g. This has an impact on recruitment and is relevant to annual stock assessment.

Due to the synchronization of male and female WCRL life cycles, metabolic reserves in the male hepatopancreas and female gonads build up during the same period (Heydorn 1969a; Cockcroft, 1997). This results in a positive relationship of male growth and female fecundity (Melville-Smith et al., 1995) and, therefore, female fecundity could be a potential predictor for male growth. Results from males and females here confirmed previous findings: A clear seasonal reserve accumulation took place in male hepatopancreas, indicated by a low HSI in austral winter that increased steadily until mid to end summer, indicating preparation for moult/growth (Chapter 3). In contrast, the course of the female HSI was not seasonal (Chapters 4 and 5), similar to earlier data (van Rooy, 1998). In both genders, indices showed inter-annual variations in extent and timing (Chapters 3 and 4). Concomitant with male HSI, female GSI increased from a low in winter a maximum in late autumn, i.e. shortly before spawning (Chapter 4; van Rooy, 1998). Both indices showed similar trends in both sampled areas, despite some differences in extent and timing (Chapters 3 and 4).

When data were arranged according to the stages in the moult cycle, a clear accumulation trend was visible in male HSI (Chapters 3 and 5) and, to an extent, in female GSI (Chapters 4 and 5). In contrast, female HSI remained relatively unchanged (Chapters 4 and 5). In the progress of the ovarian cycle, accumulation of reserves in the ovaries were clearly indicated by a steady increase of the GSI, whereas the HSI remained unchanged (Chapters 4 and 5).

In general, metabolite accumulation in male hepatopancreas and female gonads followed similar trends in accordance with relative organ size (Chapters 3,4,5). Lipid content continuously increased in both organs, however, protein concentrations were different: In male hepatopancreas, protein content displayed an inverse relationship with lipid content, because they accumulated at a much lower rate. In contrast, both metabolites increased concomitantly in ovaries. In the female hepatopancreas, there were no seasonal variations in lipid- and protein

contents, however, when analysed according to moult stage, a similar trend like in males was noticeable, although much less pronounced (Chapter 4).

Compared with palinurid hepatopancreas and ovaries, the lipid- and protein rich abdominal muscle ("tail") has previously received less research attention (Chapter 2; McLeod et al., 2004). This is also true for the WCRL. It was previously thought to reflect rather long-term changes and respond less sensitively to environmental and physiological changes (Cockcroft, 1997). However, the present research revealed some interesting results that may be worth investigating in the future. In the present study, the tail constitutes a high proportion of the total weight of a lobster (TI, measured as whole tail weight including exoskeleton) at up to 34% in males and up to 42% in females (Chapters 3,4,5). This compares to hepatopancreas sizes (HSI) of up to about 5% and ovary size (GSI) of up to about 4% (Chapters 3 and 4). In addition, the abdominal muscle constitutes by far the main portion of muscle tissue in a lobster (Munian et al., 2021). Its large size most likely makes it more difficult to detect changes. In contrast to males, where there were no signs of seasonality in TI, there were some indications in females: At its maximum, the tail is about 8% of body size larger than in males, possibly because of an involvement in reproduction as an additional metabolite storage site. Also, there is a decline in TI from ovary stage 2 onwards in both sampling areas (Chapter 4) and a 6% decline from stage 3 to 4 in Hout Bay (Chapter 5). These declines suggest a role in ovary maturation, i.e. a possible transfer of reserves to the ovaries from the abdominal muscle, where sterols and phospholipids are available in high quantities (Nelson et al., 2005; Chong et al., 2017; Munian et al., 2021). Its large size may compensate here for the tail's lower total lipid levels. Because estimations by organ size (TI) only are clearly limited in providing sufficient details, it is imperative in future to conduct biochemical research on the contribution of the abdominal muscle to growth and reproduction.

In mature male WCRL, 99% of somatic production is apportioned to growth, whereas in mature females, only 5% of somatic production is used for growth and 95% are allocated to reproduction (Zoutendyk, 1990). However, it is relatively uncertain as to how exactly metabolic reserves from the main storage sites (i.e. hepatopancreas and gonads) are partitioned between growth and reproduction in females (van Rooy, 1998), given their different reserve prioritisation (Zoutendyk, 1990). In males, where gonads are minute in size, the hepatopancreas is the only metabolic depot to fuel growth, whereas females have two (see Chapter 2), because the ovaries become an additional centre for lipid- and protein metabolism and storage during ovarian maturation (Harrison, 1990). In the present project, analysis of the partitioning of

reserves, particularly lipids, between female gonads and hepatopancreas during ovarian maturation revealed little or no reserve accumulation in the hepatopancreas prior to peak ovary reserve accumulation (Chapter 5). Ovaries build up these metabolic reserves as a supply for eggs/embryos and thus reproduction. Moreover, at any time point, females had substantially more lipids (42 – 120%) available than males (Chapter 5). Possible explanations for this difference are that females have a lower metabolic rate (permitting more fat storage), females feed on food richer in lipids than males or, females feed more than males (Chapter 5). The latter assumption is the most likely explanation because both other reasons are less likely: A female-specific lower metabolic rate (respiration) has not been reported for decapods in general, and palinurids in particular (Waldron, 1991; Crear and Forteath, 2000). Furthermore, the hepatopancreas FA profile in the present study was very similar in both sexes (Chapter 5), not indicating any dietary differences that would alter the FA profile (Smith et al., 2004; Nelson, 2005). It therefore seems that females do not need to (completely) prioritise partition of reserve between growth (ovary) and reproduction (hepatopancreas), at least in this area. However, this may be different in slow-growth areas, where food supply is suboptimal (see below).

Results from the present research allow some conclusions regarding food availability in the study areas. As mentioned above, females have substantially more lipids available than same-size males, presuming they have most likely a higher food consumption rate than males (see above). In turn, this would indicate that the Hout Bay area provides sufficient food to allow for the accumulation of metabolic reserves that are substantially higher than required for the fast growth of males recorded in the same area. Therefore, surplus food availability can be assumed to guarantee optimal male somatic growth in Hout Bay. The gender difference in total available lipids per lobster (Chapter 5) may hence be suitable to assess food availability in a specific area and, in turn, growth potential for males. A large difference would thereby indicate high growth potential for males. The Hout Bay area also seems to provide optimal food quality: The FA profile of male and female hepatopancreas and female gonads did not change during the phase of concomitant reserve accumulation (Chapter 5), as could be assumed if food quality was varying (Smith et al., 2004) and essential FA lacking (Kanazawa et al., 1979; D'Abramo, 1997). In addition, there are no changes in the FA profiles during both moult- and ovary maturation cycles in the analysed organs. The quality of available food items in this fishing area seems therefore stable during reserve accumulation and optimal for growth and reproduction. However, detailed, comparative research in different areas is required in future to proof these

assumptions. If proven correct, the gender difference in total fat stores could be suitable for assessment of food availability in a specific area and, hence, growth potential for males.

One aim of the present project was to identify potential predictors for male somatic growth (Chapter 1) because the latter is an important input into annual stock assessment procedures for the determination of the TAC (DEFF, 2020). A prerequisite for consideration as potential growth predictor is the distinct variation of a parameter over the moult cycle or season (Cockcroft, 1997). Two data sets fulfilled such seasonality in males: a) the course of the annual moult cycle and b) the accumulation of metabolic reserves for growth (Chapter 3). Whereas data arranged for the moult cycle were found unsuited, a different subset of data is potentially useful for future predictors. These are the relative hepatopancreas size (HSI), the hepatopancreas lipid content and the total amount of lipid available (i.e. per whole hepatopancreas/lobster) for the months immediately prior to moult (Chapter 3). A higher value for each of these parameters at the immediate pre-moult period in June-July (i.e. shortly before moult) correlated with a higher annual growth increment during the subsequent moult for the Hout Bay fishing area (Chapter 3). This result needs to be confirmed for more fishing areas with different historical growth increments. In contrast, present data (Chapter 3) were not robust enough to confirm previously proposed predictors from the "peak accumulation" months of March – May (Cockcroft, 1997).

A different route to predict male somatic growth is thought to be female fecundity, because a positive relationship of male growth and female fecundity for the same area exists (Beyers and Goosen, 1987; Melville-Smith et al., 1995). This is the result of the concomitant built-up of metabolic reserves in male hepatopancreas and female gonads during the same period (Heydorn, 1969a, Cockcroft, 1997). Optimal reserve built-up in the ovaries should therefore result in higher fecundity and, in turn, should indicate higher somatic growth of male counterparts. The required seasonality in females was only given for aspects of ovary maturation but not for those of moult (Chapter 4). Ovary size (GSI), ovary stage, moisture-, lipid- and protein contents were all seasonal and therefore investigated for suitability as male growth predictor. Peaks in ovary lipid content during March - May were identified as potentially useful because they indicated the point of maximum accumulation for reproduction (Chapter 4). When data for these months were analyzed separately, many parameters were found unsuited because they were too variable. The most suitable was a combination of ovary size and lipid content, calculated as total amount of lipid per ovary available for reproduction. Due to strong seasonality, this parameter seemed to have the strongest potential to predict

female fecundity and possibly male growth in subsequent spawning and male moult, respectively. Unfortunately, limitations of the sampling strategy and numerous other difficulties (such as inter-annual variation and timing of peaks) prevented a more accurate and conclusive analysis in the present study (Chapter 4). However, the study provided sufficient information to investigate this parameter further and to design a better strategy for future research.

In crabs and other decapods (but not palinurids), changes in lipid class- and FA composition of the ovary were revealed during maturation (Mourente et al., 1994). Such change could have served as an indicator for successful accumulation during preparation for male moult and female reproduction. However, no variations in the FA profile were found in male and female hepatopancreas and ovaries (Chapter 5). A reason may have been that the feeding conditions in terms of food quantity and quality (see above) in the investigated fast-growth fishing area were optimal. This may be different in areas where food supply is not optimal, i.e. where growth is slow and has to be investigated in future projects.

Although these results are not yet robust enough to qualify for the use in stock assessment, they provide important initial information for the development of a predictor for male growth increment in the subsequent moult. The project also produced important information to design a future research strategy to answer remaining questions and provide further advice for fisheries management of the WCRL resource.

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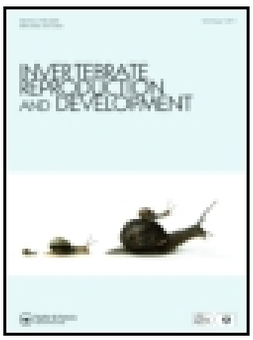

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Depot lipids in mature palinurid decapods (Crustacea)

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ABSTRACT

Decapods form the backbone of global crustacean fisheries and aquaculture. Palinurids contribute a small percentage of global decapod catches but are of high commercial value. They are currently rarely cultured but some fast-growing tropical species are regarded as one of the "most promising emerging species for aquaculture". Recent advances in palinurid aquaculture have raised interest in their metabolism, including that of depot lipids. Probably due to past difficulties in palinurid aquaculture, knowledge on lipid metabolism of adults is poor, especially when compared with commercially important aquacultural decapods. However, interpolation of data from other decapod infraorders such as penaeids, from which the bulk of knowledge on crustacean lipids is derived, is problematic. Palinurids have a completely different life cycle, demanding different metabolic requirements than related decapod taxa. Providing energy during moulting and gonad development, depot lipids play a central role in growth and reproduction of palinurids. The high relevance of lipid digestion and accumulation is indicated by the high hepatopancreas lipid content (higher than in other decapod taxa) and the increasing importance of lipids with ontogenesis. The present review intends to provide an overview of the currently available information on the role of those depot lipids in the biology of mature palinurid decapods and regulation of their metabolism.

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Introduction

Decapod crustaceans form the backbone of global crustacean fisheries and aquaculture (FAO. 2020). Palinurids (spiny or rock lobsters) contribute 28% of the 301 000 tons of total catches of all lobster groups (Penn et al. 2019) with a commercial value of more than 4 billion US $ (Radhakrishnan et al. 2019). Due to their complex larval phase and relatively slow growth rate, they were previously rarely cultured. More recently, substantial technical advances have resulted in the rapid expansion of the culture of several warm-water species in South East Asia, where a substantial industry based on growing wild-caught post-larvae (pueruli) has been established (Jones et al. 2019). In Vietnam, this industry is worth US$ 90 m per annum (Williams 2007). These developments have made palinurids one of the "most promising emerging species for aquaculture" (Phillips and Matsuda 2011) and renewed interest in understanding their metabolic processes.

Research on decapods has so far provided the bulk of knowledge on crustacean metabolism in general, including that of depot lipids (Sánchez-Paz et al. 2006). Although it is not clear as to what extent lipids are a nutritional requirement (Sánchez-Paz et al. 2006), lipids are certainly the main organic body reserve for most crustaceans (Phillips 2006). However, the preferred compound and the sequence of deposition and depletion of these lipids vary considerably between decapod crustacean species (see Sánchez-Paz et al. 2006). Glycogen (carbohydrate) reserves in crustaceans are generally limited and readily power short-term high-intensity anaerobic muscle work (such as tail-flip), after phosphagens are depleted (England and Baldwin 1983). Proteins were previously regarded as the principal energy source in crustaceans (New 1976) but more recent research revealed that crustaceans differ widely in this regard and that lipids play a major role (Sánchez-Paz et al. 2006). Lipids, as the most chemically reduced (i.e. less oxidised) organic molecules, can be stored in substantial amounts. They are catabolised to provide energy for various processes such as growth, moulting and reproduction (Sánchez-Paz et al. 2006). Also, in contrast to penaeid shrimps, proteins and lipids cannot easily be substituted by carbohydrates in palinurid feed: Spiny lobsters digest some carbohydrates well but utilise them inadequately (Rodríguez-Viera et al. 2017).

Our understanding of the lipid metabolism of palinurids is poor in comparison to that for other decapod species used in aquaculture. Due to the paucity of

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relevant palinurid data, information is often drawn from other decapod infraorders, such as the closely related Astacidae (clawed lobsters) and the Penaeidae (shrimps, prawns). However, interpolation of such data is problematic. Palinurids belong, together with Scyllaridae (slipper lobsters), to the infraorder Achelata (Wolfe et al. 2019). They have a unique and completely different life cycle (notably their complex and protracted larval phase), demanding different metabolic requirements than related decapod taxa (Perera and Simon 2015).

The high levels of depot lipids in palinurids, especially in the hepatopancreas (the main organ for lipid digestion and accumulation) indicate their importance in many biological processes (Gibson and Barker 1979). A more detailed examination of the metabolism of depot lipids in palinurids, taken into account their specific biology, is therefore desirable. This review aims to summarize currently available data on the role of depot lipids in the biology of palinurid decapods. Although the focus is on adults, reference is made to juvenile palinurids and other decapod infraorders where there are no adult palinurid data available.

Biological cycles

The biological cycles of post-larval palinurids are complex with substantial variation between species both pre and post attainment of sexual maturity (for reviews, see Lipicus and Eggleston 2000; George 2005). Moult- and reproductive cycles of males and females are mostly seasonal and tightly coupled in some species (see below). At the physiological level this is reflected in depot lipid dynamics of both sexes. Adult males moult well before females and are in intermoult (hard) stage (C) at mating (Lipcius 1985). Mating takes places a certain period after female ecdysis but the period differs from a few days to weeks between species (Lipicus and Eggleston 2000).

The Southern African silent *Jasus lalandii*, for example, adopts an annual moult cycle after attainment of sexual maturity (Newman and Pollock 1974), after which male and female moult- and reproductive cycles are tightly synchronised. Adult male *J. lalandii* moult in late austral spring to early summer (September–December), well before females in late autumn to early winter (April-June) and the mating period in winter (July–August). At the time of oviposition and spawning, the female exoskeleton is still relatively soft (Heydorn 1965, 1969; Berry and Heydorn 1970; Zoutendyk 1990; Dubber et al. 2004) and pleopodal setae are fresh for egg attachment (Booth 2006). As a result of this synchronisation, lipid- and other metabolic reserves in the male hepatopancreas and female gonads build up during the same period in *J. lalandii* (Heydorn 1969; Cockcroft 1997) and a relationship of male growth and female fecundity was shown (Melville-Smith et al. 1995). Environmental influences, therefore, impact both simultaneously (Cockcroft 1997), most likely via affecting the build-up of lipid depots.

The substantial energetic cost of reproduction is the major difference in the energy budgets of adult males and females. Until they reach maturity, juvenile males and females have similar growth rates. Thereafter, the females' moult increment is reduced to provide more energy for gonad production. In large reproductive *J. lalandii* females, only 5% of somatic production is used for growth and 95% are allocated to reproduction (Zoutendyk 1990). Gonad size of males is small and therefore most of somatic production is apportioned to growth (99% in *J. lalandii*, Zoutendyk 1990). In times of nutritional stress *J. lalandii* females sacrifice growth to optimise egg production (Cockcroft 1997). As detailed below, the main metabolite to fuel growth and reproduction in palinurids are lipids.

Lipids stored in palinurid decapods

Like other decapods, palinurids contain high concentrations of lipids that are mainly concentrated in the hepatopancreas, ovaries and abdominal muscle (see Table 1 for an overview). Lipid concentration in some of these tissues varies with moult- and reproductive cycles. The dominant lipid classes (see Figure 1 for structures) are triacylglycerols (TAG), phospholipids (PL) and sterols (ST).

Triacylglycerols

There is very little information on TAG contents and dynamics in mature palinurids but data from post-larval animals (i.e. pueruli and juveniles) reveal that the overall concentration increases during ontogenesis until TAGs (Figure 1(a)) become the dominant lipid class (Jeffs et al. 2002). In the pelagic stages, TAG content is very low and instead, PLs in the fat body (located laterally in the haemocoel) serve as depot lipid for energy provision. This seems to be an adaptation to a pelagic/nectonic life where translucent PLs ensure camouflage in contrast to the opaque TAGs (Jeffs et al. 2001). After settlement, PLs from the fat body are rapidly converted to TAG and deposited in the developing hepatopancreas for the moult to juvenile stage (Jeffs et al. 2001).

Table 1. Overview of depot lipids in the main storage organs of palinurid crustaceans.

	Total Lipid content*	Lipid composition	
	(% dry weight)	Lipid classes (% of total lipids)	Fatty acid profile (dominant FAs)
Hepatopancreas	max. 70–75%	TAG: 90% ST: < 1% PL: < 1.7%	Saturated and unsaturated long-chain FA
Abdominal muscle	0.2–2%	TAG: 1% ST: < 1% PL: ~ 5–8%	Saturated and unsaturated long-chain FA
Ovaries	~40%	TAG: 45% ST: < 3–4% PL: 50%	Saturated and unsaturated long-chain FA

See text for more details and references. *Please note: These values fluctuate during biological cycles and respective maximal values (depending on reproductive- and moult cycles). Abbreviations: TAG – triacylglycerols, ST – sterols, PL – phospholipids, FA – fatty acid.

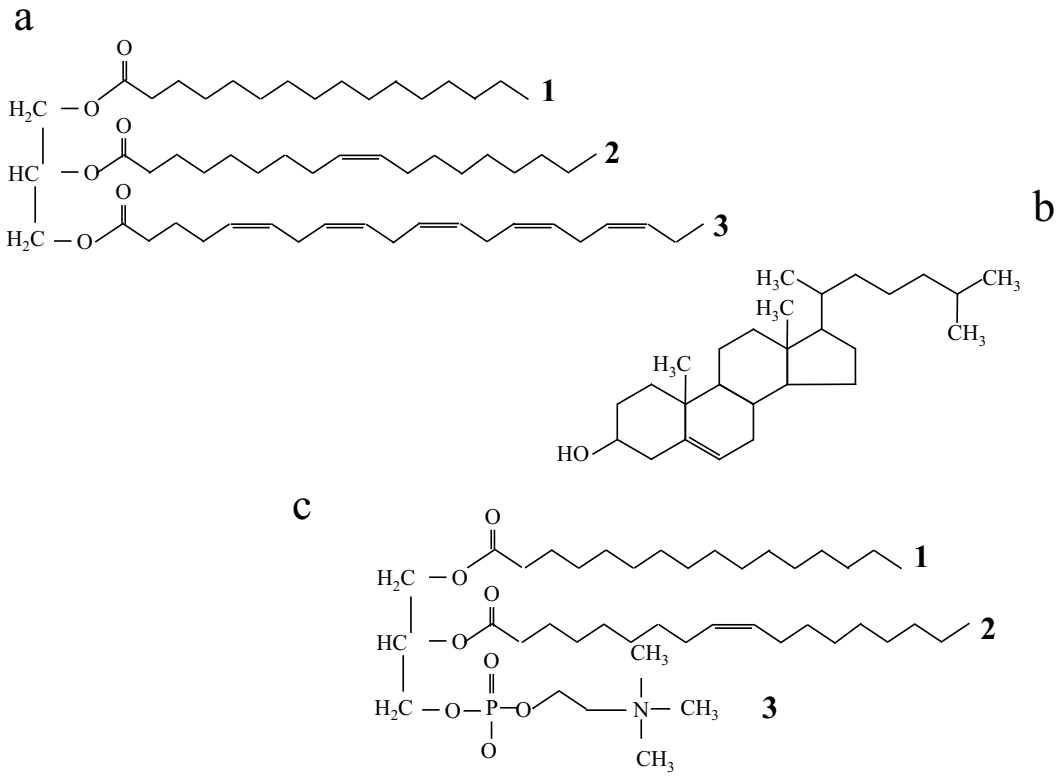

Figure 1. Primary chemical structures of main depot lipids in palinurids: (a) a Triacyl-glyceride with dominant fatty acids found in palinurids as residues 1) palmitic acid (C16: 0), 2) oleic acid (C18: 1 and 3) EPA (C20: 5 n); (b) Cholesterol (the dominant sterol) and (c) Phosphatidyl choline, the dominant phospholipid, with the saturated fatty acid 1) palmitic acid and the unsaturated fatty acid 2) oleic acid as residues and 3) the phosphate group with choline residue. Note: Secondary and tertiary structures not taken into consideration.

Sterols

Cholesterol (Figure 1(b)) constitutes 90–95% of total sterols in crustaceans (Kanazawa 2001) and is organ-specific in palinurids (Smith et al. 2004). It is also the dominating sterol in *J. lalandii* hepatopancreas with only traces of other sterols present (Ligthelm et al. 1953). Cholesterol plays an important role as a cell constituent and as a metabolic precursor of steroid hormones and moulting hormones (Harrison 1990). Spiny lobsters are, like crustaceans in general, unable to synthesise sterols *de novo* but can convert some sterols to cholesterol (Teshima and Kanazawa 1971) and therefore need to obtain sterols from their diet: A lack of dietary cholesterol was shown to lead to mortality in clawed lobsters when dietary inclusion was too low (Kean et al. 1985). Dietary inclusion of 0.2–2.0% cholesterol is regarded as optimal for a range of crustacean species (Kanazawa 2000).

Phospholipids

In general, the polar PLs (Figure 1(c)) are components of membrane structures and mediators and modulators of transmembrane signalling. In crustaceans, certain phospholipids are necessary as constituents of lipoproteins (High-density lipoproteins (HDL) and very high density lipoproteins (VHDL)) which play an important transport role during mobilisation of lipids to and from the hepatopancreas (Lee and Puppione 1978; Kanazawa et al. 1985; Yepiz-Plascencia et al. 2000, 2002). Phosphatidylcholine is a particularly important PL because it is an essential component of these lipoproteins (Hertrampf 1992). Phospholipids play a critical role in lobster growth and development, which is attributed to the lobsters' (crustaceans in general) limited ability to biosynthesize phospholipids from fatty acids (Kanazawa et al. 1985). Lobsters therefore need to obtain PLs from their diet. Phosphatyl Choline (PC) is the dominant PL in *J. lalandii* hepatopancreas with a ~ 60% content and ~71% in ovaries (de Koning and MacMullan 1966).

Fatty acid composition of storage lipids

The fatty acids (FA) α-linolenic acid (ALA, 18:3 n-3), linoleic acid (LA, 18:2 n-6), Eicosapentaenoic acid (EPA, 20:5 n-3), Docosahexaenoic acid (DHA, 22:6 n-3) and Arachidonic acid (AA, 20:4 n-6) are essential fatty acids (EFA) for growth and survival of crustaceans in general and cannot be synthesised *de novo* (Kanazawa et al. 1979; D'Abramo 1997; Querijero et al. 1997; Glencross and Smith 2001). There is little information about the role and function of these specific EFA in spiny lobsters (Williams 2007; Glencross 2009), however, during starvation of post-puerulus *Panulirus cygnus* (DHA, EPA, AA; Limbourn et al. 2008) and ontogenesis of *Jasus verreauxi* (post-larvae, LA, DHA, EPA; Jeffs et al. 2002), these EFA are spared from catabolism at the cost of non-essential FA. The same seems to be true in the hepatopancreas and the leg muscle during increased metabolic activity of *J. edwardsii* due to environmental change (EPA, Chandrapavan et al. 2009). In *J. lalandii* hepatopancreas, the main fatty acids are saturated C:14, C:16, C:18, C:20, C:22 and unsaturated C:14, C:16, C:18, C:20, C:22, C:24, C:26 (Ligthelm et al. 1953). Long chain fatty acids also dominate the fatty acid profile of ovarian lipids (Smith et al. 2004).

Dietary sources of storage lipids

Palinurids selectively prey on a diverse assemblage of benthic and infaunal species such as molluscs (gastropods, bivalves), other crustaceans (including crabs, barnacles, mysids), echinoderms, polychaetes, coralline algae and sponges (Barkai and Branch 1988; Lipcius and Eggleston; Lipicus and Eggleston 2000). Cannibalism is also relatively common among palinurid lobsters (Barkai and Branch 1988; Booth and Kittaka 1994). Dominant components of the diet of most palinurid species seem to be mytilid mussels (genera *Mytilus, Aulacomya, Perna*), sea urchins and other crustaceans. The ribbed mussel *Aulacomya atra*, for example, can constitute up to 97% of the stomach content of *J. lalandii* (Pollock 1979). In many experiments, feeding mussels achieved maximum growth and lipid accumulation in the hepatopancreas when compared with all other natural and artificial diets (Glencross et al. 2001; Esterhuizen 2004; Ward and Carter 2009; Simon and Jeffs 2013). The abundance and distribution of mussels has been directly linked to lobster growth rates in the field (Beyers and Goosen 1987; Field and Wickens 1988). A reason for the importance of mytilid mussels as dietary source for palinurids is, apart from their high total lipid- and phospholipid content, their high content of some long-chained polyunsaturated fatty acids such as EPA, DHA and ARA (Kariotoglou and Mastronicolis 1998). These dietary fatty acids are essential for development and growth of the lobsters during critical stages of their growth cycle (see above, Esterhuizen 2004). Sea urchins are also a rich source of nutritional lipids: Urchin gonads are not only regarded as a reproductive organ but also a metabolite storage site that contributes 10–15% of total biomass and contains about 5% total lipids, which are rich in essential FA such as DHA, ARA and EPA (Liyana-Pathirana et al. 2002; Arafa et al. 2012). Crustacean prey species also provide a rich source of dietary lipids.

Lipid storage sites

Major storage sites of depot lipids in decapods, including palinurids, are the hepatopancreas, female gonads and muscle tissue (see Table 1). The amount and types of lipid stored change during the moult and reproductive cycles (see, for example, Harrison 1990; Sánchez-Paz et al. 2006). These changes are interlinked, are impacted by environmental influences (including food availability), and are under endocrine control.

The Hepatopancreas (or digestive gland), is a central organ in the biology of decapods and serves vital metabolic functions such as enzyme synthesis and secretion, uptake and storage of nutrients and their precursors, synthesis and catabolism of storage metabolites, excretion, provision of material and energy for moult and growth (Gibson and Barker 1979). It is typically high in lipids and is the principal organ for their digestion, absorption, storage, synthesis and distribution (Gibson

and Barker 1979). The palinurid hepatopancreas has a particularly high lipid content that can reach 70–75% of dry weight in the late pre-moult stage (Cockcroft 1997; McLeod et al. 2004; Smith et al. 2004). Relative hepatopancreas size and its lipid levels respond rapidly to changes in physiological and environmental parameters. Lipid depots are used up quickly in response to environmental and physiological changes such as moult (Cockcroft 1997) and dietary stress (Smith et al. 2004).

The palinurid hepatopancreas starts to form after settlement, when PL from the so-called fat body of the puerulus (post-larva) are converted into TAG and moved to the hepatopancreas for storage and mobilisation during the subsequent moult (Nishida et al. 1995; Pearce 1997; Jeffs et al. 2001). The fat body seems to become redundant thereafter. In adult spiny lobsters, the main lipid class in the hepatopancreas are the TAGs (Ligthelm et al. 1953; Smith et al. 2004) where their contribution can be as high as about 90% of total lipids (TL)(Smith et al. 2004). TAGs of the hepatopancreas seem to be the preferred lipid class for energy provision: Their share declined concomitantly with a decline in TL during starvation (Smith et al. 2004). In contrast to other organs, ST concertation is less than 1% in palinurid hepatopancreas (Smith et al. 2004). The principle ST in *J. lalandii* is cholesterol (Ligthelm et al. 1953). PL are also very low (0.4–1.7%) (Ligthelm et al. 1953; de Koning and MacMullan 1966; Smith et al. 2004). Phosphatyl Choline (PC) is the dominant PL in *J. lalandii* hepatopancreas (~60%)(de Koning and MacMullan 1966), playing an important role in the survival and growth of the lobster. This is attributed to selective fatty acids such as linoleic-, linolenic-, EPA- and DHA fatty acid groups (Koshio and Kanazawa 1994).

In decapod hepatopancreas, lipids are at their minimum immediately after ecdysis and are starting to accumulate again at the end of the postmoult (stages A-B) through intermoult up to stage C_4 and onset of premoult (D_1). Later in the premoult stage (from D_2) lipid levels decrease rapidly, coinciding with non-feeding in stages $D_{2'}$–B around ecdysis (Charmantier-Daures and Vernet 2004). Lipids therefore built up in the hepatopancreas in preparation of the moult until the late intermoult (lipid peak) phase after which reserves are used/redistributed for processes related to ecdysis. In male *J. lalandii*, the peak lipid storage period has a positive correlation to the measured growth (moult) increment and can be used as a reliable potential indicator for lobster growth rate (Cockcroft 1997). A lack of lipid accumulation during intermoult by this species was shown to lead to low growth rates and even negative growth (shrinkage) (Cockcroft and Goosen 1995; Cockcroft 1997). The relative size of the hepatopancreas (Hepatosomatic Index = HSI) changes mainly with the dynamics of depot lipids: Both peak around July to August in male *J. lalandii*, i.e. shortly before male moult (Cockcroft 1997).

Studies on the lipids in organs of female palinurids have received less attention. The timing and amount of the lipid present in the female hepatopancreas differs from that of males. Lipid levels remain low during ovarian maturation and peak shortly after HSI reaches its maximum. They remain low during the berry cycle and recover only slightly thereafter. In *J. lalandii*, this is in July and October to January, respectively (Van Rooy 1998).

Female Gonads (Ovaries)

Whereas male gonads are very small, and negligible as an energy depot, the ovary becomes an additional centre for lipid metabolism during ovarian maturation in decapod crustaceans including palinurids. Lipids accumulate together with proteins and the GSI (Gonadosomatic Index) increases (Harrison 1990). Lipids in gonads accumulate until spawning and the GSI increases concomitantly (Van Rooy 1998). As a result, ovaries contain high concentrations of total lipids (TL) reaching approximately 12.5% (wet weight) in *J. lalandii* (de Koning and MacMullan 1966) and 41% (dry weight) in *J. edwardsii* (Smith et al. 2004). TAG contribute approximately 45% of TL whereas ST content (~3-4% of TL) is higher than in hepatopancreas but lower than in abdominal muscle (Smith et al. 2004). The increased storage of ST (cholesterol) during maturation most likely serves incorporation into egg- and embryo membranes (Harrison 1990). Palinurid ovaries are particularly high in PL (about 50% of TL, Ligthelm et al. 1953; de Koning and MacMullan 1966; Smith et al. 2004) which comprise mostly of PC (~71%) and phosphatidylethanolamine (PE, 23%) whilst their fatty acid profile consists mostly of long chain fatty acids such as palmitic acid (16:0) DHA (22:6) and EPA (20:5) (de Koning and MacMullan 1966).

Dynamics of lipids in decapod ovaries and hepatopancreas are interlinked. The lipid accumulation in decapod ovaries is a result of vitellogenesis (production of vitellogenin and lipoprotein and storage by oocytes) in the ovaries themselves but possibly also with contribution from the hepatopancreas from where it is subsequently transferred to the ovaries via the haemolymph (Harrison 1990). In palinurids, however, there is indirect evidence for *J. edwardsii* that lipid demand of ovarian maturation is to a large extent met through dietary intake and not only (or not at all) from the hepatopancreas (Smith et al. 2004) as it was previously found in several crab species (Hasek and Felder 2005, 2006). Changes in the composition of lipids concerning lipid

classes and FA during maturation, such as documented for crabs (Mourente et al. 1994), are yet unknown for palinurids. Ovarian maturation and reserve built-up depends on food supply: Starvation leads to reduction of gonad index (GSI) and lipid content in *J. edwardsii* (Smith et al. 2004).

Abdominal muscle

Although the abdominal (or tail) muscle is considered an energy reserve in most crustaceans, it is not utilised as a major energy source by male *J. lalandii* during a routine (non food-limited) moult cycle. The lipid content is low between 0.2 and 2% and changes little during the growth periods (Cockcroft 1997). It was therefore concluded that abdominal muscle reflects more long-term changes and responds less sensitively to environmental and physiological changes experienced in the field (Cockcroft 1997). In adult male *J. edwardsii*, however, lipid content almost halved while proteins remained constant after a 28-day starvation period (McLeod et al. 2004). This indicates a lipid storage function of the abdominal muscle and the consumption of lipids to preserve (structural) proteins during periods of extended nutritional stress. The tail muscle constitutes a high portion of the total mass off a palinurid, for example 45% (with exoskeleton) in *P. gilchristi* (Groeneveld and Goosen 1996). This compares to a maximum 5% of the hepatopancreas at peak accumulation in most palinurids. Therefore, the total amount of lipids in the tail should not be neglected. In contrast to the hepatopancreas, where TAG form the bulk of TL, the tail is dominated by PL with levels of about 90% (Smith et al. 2004; Nelson et al. 2005; Chong Shu-Chien et al. 2017) and TAG content is low at about 1% (Smith et al. 2004). In addition, sterol concentration is highest in abdominal muscle (5–8% in *J. edwardsii*; Smith et al. 2004). It can therefore possibly be regarded as a cholesterol storage site as suggested for prawns (Kanazawa et al. 1988).

Metabolism

Before any dietary lipids can be metabolised, food items of spiny lobsters are masticated by their mouthparts before they are ingested into the foregut. The latter contains a gastric mill that further crushes and mixes food particles (Johnson and Hooper 1992). The volume of the foregut is only 2–3% of body weight and hence, food consumption is limited to an estimated 1 foregut volume of food per day (Perera and Simon 2015). Quality food is therefore essential for optimum growth and reproduction and may explain the high ratio of mytilids in the diet of spiny lobsters as they have a high lipid content and are rich in essential FA and phospholipids. Lipid digestion (TAG and PL) is facilitated by gastric lipases produced and secreted by the F-cells of the hepatopancreas, although these lipases have not been characterised so far in terms of triglyceride hydrolase- and phospholipase activities (Perera and Simon 2015). High content of PLs is positive due to their high solubility and digestibility and PLs may also enhance transport and retention of TAG and ST (Coutteau et al. 1997). In the gastric space, neutral lipids are cleaved into mono- and diglycerides and then converted to phospholipids by the B-cells of the hepatopancreas and absorbed together with FA (Chang and O'Connor 1983; Ceccaldi 2006). From here, they are released and transported in the haemolymph for use in organs or for conversion to TAG for storage (Chang and O'Connor 1983). Phospholipids are the main circulating lipids in crustaceans (Chang and O'Connor 1983). This is due to their role as part of lipoproteins: HDL and VHDL play an important role in the transport of lipids during mobilisation of lipids to and from the hepatopancreas (Lee and Puppione 1978; Kanazawa et al. 1985; Yepiz-Plascencia et al. 2000, 2002). Phosphatidyl Choline is a particularly important PL because it is an essential component of these lipoproteins (Hertrampf 1992). For energy production, first-step enzymes (lipases, esterases) mobilise stored TAG and PL. These enzymes are usually under endocrine control; however, exact details on such regulation in decapods are unknown as yet. Fatty acids liberated from these TAGs and PLs are catabolised via beta oxidation in the target tissues, such as muscle. Activity of the beta oxidation enzyme HOAD (3-hydroxyacyl-CoA dehydrogenase) is positively correlated with the increasing role of lipid stores during ontogenesis in *J. edwardsii* (Wells et al. 2001).

Endocrine control

The dynamics of depot lipids in palinurids are driven by food availability and are under endocrine control. In general, moulting (i.e. growth) and reproduction in decapod crustaceans are under complex endocrine control by respective antagonistic hormones. Briefly (and not comprehensively): Ovarian maturation, including vitellogenesis, is stimulated by the release of the gonad-stimulating hormone (GST) from brain and thoracic ganglion, synthesis of which is inhibited, in turn, by vitellogenesis-inhibiting hormone (VIH) produced in and released from the sinus glands of the eye stalks (Nagaraju 2011). So far, no unique structures of a VIH

(of the cHH/MIH/VIH group of peptides) has been found in palinurids (Marco and Gäde 2006) but VIH activity was shown for known cHHs of *J. lalandii* (Marco et al. 2002).

Moulting, which is necessary to adjust the exoskeleton to a larger body and allow for growth, is initiated by steroid moult hormone (ecdysteroid) that is produced in the Y-organ and whose antagonist is the moult-inhibiting hormone (MIH) produced in the sinus glands (Carlisle and Knowles 1959). A palinurid ecdysone (20 α-hydroxy ecdysone) was discovered in *J. lalandei* (now *J. edwardsii*; Passano 1960; Hampshire and Horn 1966) and *J. lalandii* (Marco et al. 2001), whereas structures of MIH in palinurids are known for *J. lalandii* and *J. edwardsii*, respectively (Marco et al. 2000a, 2001; Christie and Yu 2019). Endocrine control of growth and reproduction also determine the lipid aspect of these two processes. Suppression of moult by MIH allows for the accumulation of lipids in the hepatopancreas, whereas GST allows for the synthesis and accumulation of lipids in the ovaries during the respective phases in the moult- and ovarian cycles. There is no published information to date on the direct endocrine control of lipid synthesis or catabolism by these hormones in palinurid crustaceans, or in fact in decapods in general.

Food consumption in palinurids is of course regulated by the availability of food items of sufficient quality and quantity (Perera and Simon 2015). Food intake in decapods is also under hormonal control by the neuropeptide NPF which was shown to increase food intake, leading to faster growth, in penaeid shrimps (for review see Christie 2011). Two members of the NPF family were recently found in *Sagmariasus verreauxi* and *J. edwardsii* but an effect on food uptake has yet to be demonstrated (Ventura et al. 2014; Christie and Yu 2019). In addition, eyestalk ablation, elimination of MIH and CHH (see above), increase food intake and growth in the spiny lobsters *Panulirus homarus* (Radhakrishnan and Vijayakumaran 1984; Vijayakumaran and Radhakrishnan 1984) and *P. ornatus* (Juinio-Meñez and Ruinata 1996). This suggests a role of eyestalk neuropeptides in regulation of food intake, since NPF seem to be produced by various nervous organs (Christie 2011).

Members of the so-called hyperglycaemic hormone family (cHH) were shown to mobilise carbohydrates in palinurids (Marco et al. 1998, 2000b; see Figure 2 for structure); however, hyperlipaemic activity was only shown so far in three infraorders of decapods: crabs, freshwater crayfish (Santos et al. 1997) and shrimps (Camacho-Jiménez et al. 2017; Montiel-Arzate et al. 2020). Haemolymph levels of Total Lipids, TAG, PL and FA were positively affected. In addition, in a freshwater crayfish, cHH caused the release of PL and FA from hepatopancreas *in vitro* (Santos et al. 1997). A similar hyperlipaemic activity of the known cHH peptides is likely in palinurids but has not yet been reported.

Conclusion

Despite their importance as a fisheries resource and the increasing importance associated with their aquaculture potential, there is no coherent body of information on all aspects of depot lipid metabolism in mature palinurids. Metabolism of depot lipids is a central aspect of palinurid biology and this review highlights the relative dearth of studies and information on this important topic. Comparison of available information on lipid metabolism in mature palinurids is confounded by a number of difficulties including research conducted at different stages of the moult and reproductive cycles, different units of measure and research objectives. The tightly-coupled biological cycles of palinurids make comparable research and data analysis extremely challenging. Future research should aim at establishing a consistent body of data covering the dynamics of depot lipids in the various relevant organs of palinurid species. Among important single topics to investigate is the contribution of the hepatopancreas towards ovarian maturation as well as changes in the composition of the

a

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Jala cHH-I:  AVFDQSCKGVYDRSLFSKLDRVCDDCYNLYRKHYVATGCRRNCYGNLVFRQCLDDLMLVDVVDEYVASVQMV-NH2
Jala cHH-II: AVFDQSCKGVYDRSLFKKLDVVCDDCYNLYRKPYVATGCRENCYSNLVFRQCLDDLMLVDVVDEYVSTVQMV-NH2
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b

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Jaed cHH-I:  AVFDQSCKGVYDRSLFKKLDVVCDDCYNLYRKPYVATGCRENCYSNLVFRQCLDDLMLVDVVDEYVSTVQMV-NH2
Jaed cHH-II: AVFDVACKGFYDRKIWAKLNRACEDCQNLYRKPGLDVDCRKGCFATPIFPMCVGELQLPVKEYKALAVLVR-NH2
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Figure 2. Primary structures of cHH hormones, potentially involved in lipid metabolism in the palinurids (a) *Jasus lalandii* and (b) putative hormomes in *Jasus edwardsii* derived from BLAST analysis of genome.

lipids (lipid classes, FA profile) during the various life cycles. Such data would not only help to establish condition indicators for the fishery but also help in optimising growth and reproduction output in palinurid aquaculture.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Analysis of biological and biochemical parameters of adult male spiny lobsters *Jasus lalandii* for identification of possible growth predictors

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ABSTRACT

Biological body parameters of adult male West Coast rock lobsters, *Jasus lalandii*, of commercial size were analysed over two years to determine reserve accumulation during two moult (i.e. growth-) cycles. Two fishing areas were compared that were historically known for fast- and slow growth, respectively. In addition, setagenic development for moult stage determination and the biochemistry of the hepatopancreas were investigated from the fast growth area. This information was intended to confirm previously proposed predictors of annual male moult increment and identify potential new ones. Annual moult increments in these areas were determined from tag-and-recapture studies, including the seasons for which reserve accumulation was analysed. Data from the present study were not robust enough to confirm previously proposed predictors from peak accumulation months (March – May). Data arranged per moult stage were also unsuitable for prediction. Further results analysis, however, identified a subset of data that are potentially more useful: Hepatosomatic index HSI (+ 6 %), lipid content (+ 9 % w_w, + 12 % w_D) and lipid per whole hepatopancreas (+ 24 %) are all significantly higher in July 2011 than in July 2010, the last sampled months before the respective annual moult. These differences correlate with a 26 % (0.7 mm) faster growth during subsequent moult in 2011 than in 2010. Aspects of hepatopancreas lipid metabolism in the months prior to moult have therefore the potential to provide simple and robust predictors of growth in adult male *J. lalandii*. However, more research is required that also includes the months adjacent to July in more than one area and for a longer period than two years.

1. Introduction

The West Coast rock lobster (WCRL), *Jasus lalandii,* is a slow-growing, cold- to temperate water palinurid species from the southern African Atlantic coast. Its fishery is one of the most important in South Africa due to its high value (2016 = R 538 m; ~$40 m) of which 98 % is exported (Fishing Industry Handbook, 2018). Additionally, the fishery is an important provider of employment for about 4 200 people, most of them along the South African west coast where impoverished communities live (DEFF, 2020). The Branch: Fisheries of the respective national governmental Department (currently Department of Environment, Forestry and Fisheries - DEFF) manages the West Coast rock lobster (WCRL) resource per zone (Fig. 1) by means of Total Allowable Catch (TAC) and, more recently, by additional effort control. Other management measures include a defined fishing season and fishing areas, a minimum legal size and a ban on the retention of berried females (Cockcroft and Payne, 1999). Despite these measures, the resource remains under heavy fishing pressure (including substantial poaching) and is heavily depleted (DEFF, 2020). The current harvestable portion of the resource (males above 75 mm carapace length) is estimated to be only 1.8 % of pristine levels (DEFF, 2020). Accordingly, annual catches have declined from a peak of 18 000 t in the early 1950s to about 1 000 t in 2019 (DEFF, 2020). In addition to fishing pressure, environmental influences have impacted the resource including reduced growth rates (Pollock et al., 1997), low-oxygen events that caused mass stranding of lobsters (Cockcroft, 2001) and an eastward shift in lobster distribution (Cockcroft et al., 2008).

To achieve sustainability and rebuild the stock, an operational management procedure (OMP) was introduced in 1997 (Cockcroft and Payne, 1999). The OMP uses abundance indices (commercial Catch per Unit Effort (CPUE) and a fisheries independent abundance index derived from annual monitoring surveys (FIMS)) and male somatic growth rate

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to set the TAC prior to the start of the fishing season (DEFF, 2020). While the physiological condition of lobster is taken into account indirectly by the OMP, the ability to predict male somatic growth rate using a reliable physiological index would be a valuable improvement to the TAC setting process. This would be novel not only in South African fisheries but also worldwide where the use of physiological indicators as input data for setting catch levels is scarce.

Such indicators can be organ- or body part indices (in relation to each other or to total weight) and biochemical composition of organs. However, such indices are very species- and life stage-specific. Attempts have been made, for instance, to use the ratios of lipid classes (triglycerides vs. cholesterol) in larval American (clawed) lobsters (Harding and Fraser, 1999), the refractive index of haemolymph and weight to carapace length ratio in juvenile *Jasus edwardsii* (Oliver and MacDiarmid, 2001). Several parameters have been investigated to predict male moult increment in *J. lalandii*, such as hepatopancreas- and abdominal muscle size and composition, as well as weight to carapace length ratio (Cockcroft, 1997). Results suggested that aspects of biochemical hepatopancreas composition (moisture, lipids, proteins) are promising candidates.

Mature *J. lalandii* moult and reproduce once a year (Pollock, 1986). Males moult in late spring to early summer (September-December), well before females in late austral autumn to early winter (April-June) and the mating period in winter (July-August)(Heydorn, 1969a). In males, energy accumulation takes place between the annual moulting events and relies on sufficient prey (Cockcroft, 1997). They use 99 % of energy reserves accumulated during intermoult for growth, in contrast to females which use 5 % (Zoutendyk, 1990). While the maturing ovaries of females become a second center of energy accumulation (Harrison, 1990), large energy reserves in males are stored in the hepatopancreas or midgut gland (Cockcroft, 1997). Due to its large relative size (~30 % of body weight), though, the storage capacity of the abdominal muscle ("tail") is also substantial (Smith et al., 2004). However, metabolite concentrations in the latter are seasonally less changeable than in the hepatopancreas (Cockcroft, 1997). Main energy metabolites are lipids and proteins but although proteins were previously regarded as the principle energy source in crustaceans (New, 1976), more recent research revealed that crustaceans differ widely in this regard and that lipids play a major role (Sánchez-Paz et al., 2006). Carbohydrate reserves (in the form of glycogen) play a minor role: they are limited in crustaceans and only power short-term high-intensity anaerobic muscle work, such as tail-flip, after phosphagens are used up (England and Baldwin, 1983). In addition, carbohydrates provide structural chitin building blocks (N-acetylglucosamine) for the formation of the exoskeleton, which are not available for energy production (Charmantier-Daures and Vernet, 2004).

As in other decapods, the hepatopancreas is particularly relevant for many biological processes in palinurids and it is their main organ for lipid digestion and accumulation (Smith et al., 2004; Perera and Simon, 2015; Munian et al., 2020). Its lipid content is therefore high but varies with the moult cycle (Cockcroft, 1997). The lipid deposits in the hepatopancreas serve growth and the formation of the new exoskeleton. Therefore, the peak lipid storage period has a positive correlation to the measured growth increment and was proposed as a potential reliable indicator for growth in males, whereas for proteins, no such relationship was found (Cockcroft, 1997). A reduced level of lipid accumulation during intermoult by the species was shown to lead to low growth rates and even shrinkage (Cockcroft and Goosen, 1995; Cockcroft, 1997). Since reserve accumulation in male WCRL is almost exclusively aimed at providing energy and structural metabolites for moulting, it is helpful to link these processes to distinct stages in the moult cycle. Moult stage was previously determined by the subjective hardness of the exoskeleton and

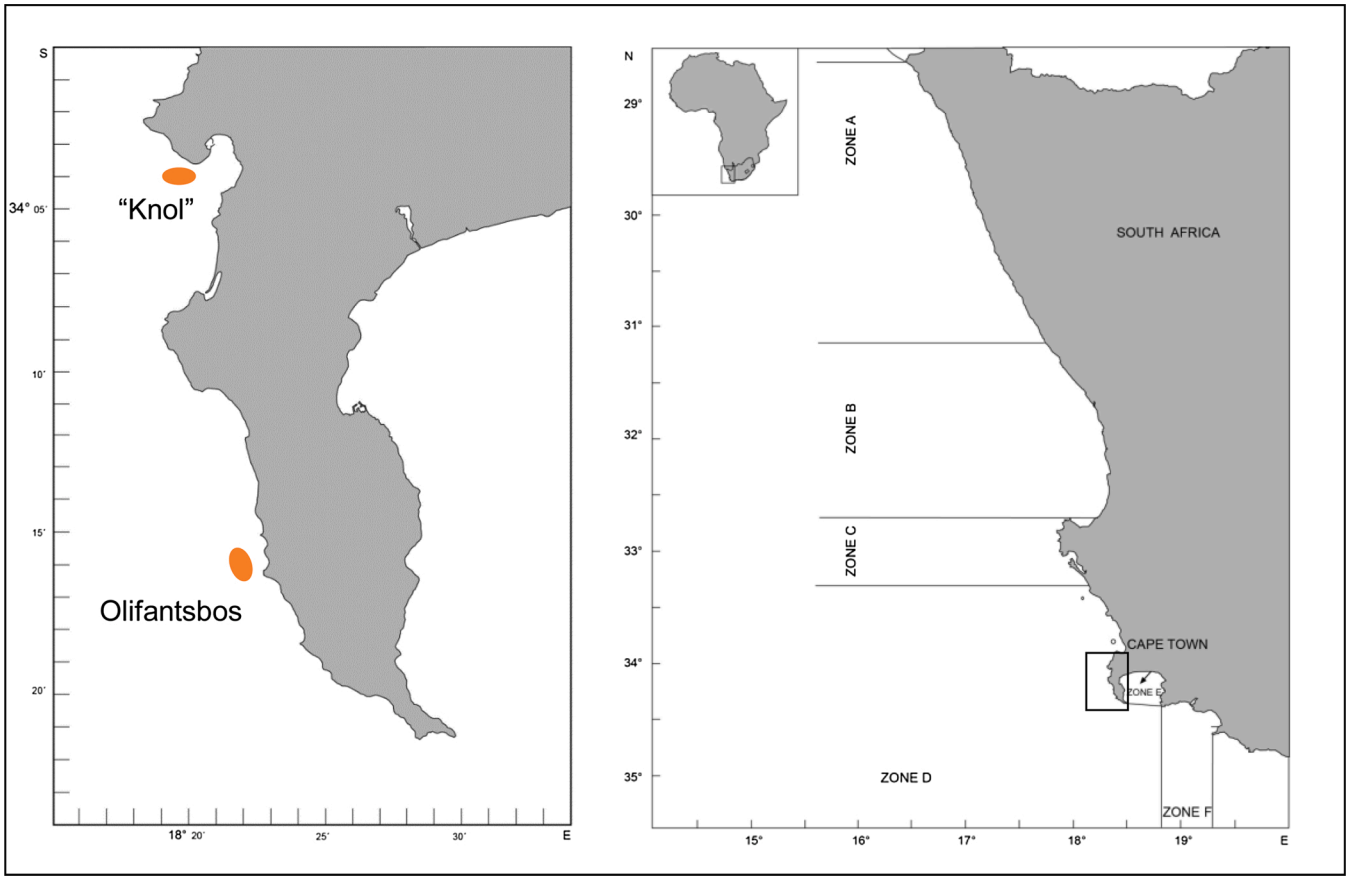

Fig. 1. Study area, showing the two sampling sites "Knol" in Hout Bay and Olifantsbos (orange).

divided into four "hard shell- and soft shell states" (Heydorn, 1969a). This is more practical but less exact than analysis of titres of moulting hormone or setagenic analysis (Marco, 2012). Additional information on these processes in *J. lalandii* throughout fishing- and moulting seasons would aid understanding of what determines growth increment at the next moult. This is because the current state of knowledge on direct and indirect environmental factors, such as food availability, that impact growth and reproduction of the WCRL is, as described above, suboptimal for predicting population- and resource development and, in turn, sustainable management. In particular, the impact during the reserve accumulation phase is important, because starvation or sub-optimal food supply may impact biological events (moult, spawning) long before they happen.

Historically, growth rates of WCRL of commercial size differ from area to area (Cockcroft and Goosen, 1995). It was found, for example, that there is a substantial difference between two catch areas in close proximity along the Cape Peninsula (about 30 km apart): Olifantsbos and Hout Bay. In Olifantsbos, growth rates have been substantially lower than in Hout Bay since the early 1990s and the two sites were therefore compared previously (Cockcroft, 1997). Such regional growth differences are assumed to be caused by variations in food availability and composition (Newman and Pollock, 1974; Melville-Smith et al., 1995; Chandrapavan et al., 2009, 2010).

The aims of this study were to determine and compare the seasonal variation in the biological cycles (moult and somatic growth rate) and biochemical composition of male lobsters from a high growth (Hout Bay) and low growth (Olifantsbos) area and to determine the potential for the development of a reliable physiological index to predict male somatic growth.

2. Materials and methods

Male rock lobsters (n = 24–30 per sampling) of 65–82 mm carapace length (CL) were sampled more or less monthly, depending on availability of ship's time and sea conditions, at two sites on the South African west coast (Fig. 1) from March 2010 to March 2012, covering two moult cycles. The Hout Bay site ("Die Knol"; 34°04'S, 18°20'E), located in a rock lobster sanctuary, is historically a fast growth area, whereas the Olifantsbos site (34°16'S, 18°22'E), in a commercially exploited area, is characterized by slow lobster growth rates (Cockcroft, 1997). The official fishing season in both areas spans from mid-November to July. Lobsters were sampled from the research vessel RV "Ellen Khuzwayo" with the aid of standard commercial gear (Pollock, 1986) at depths of approximately 80–200 m. After capture, total wet weight (w_T) and carapace length (CL) were measured; w_T was determined by digital balance to the nearest g, whereas Vernier calipers were used to measure CL (from the tip of the rostrum to midpoint of posterior edge of carapace) to the nearest 0.1 mm. Animals were subsequently sacrificed and dissected. Abdominal muscle (tail) wet weight (w_A) was determined after dissection, whereas hepatopancreas was transferred into pre-weighed vessels. Samples were then blast-frozen aboard immediately and later transported to freezers at the laboratory where they were kept at −40 °C for later weight determination and biochemical analysis (Hout Bay samples only). These samples remained sealed from air in a plastic vial to prevent auto-oxidation of the lipids in the sample. For determination of moult stage, a pleopod was sampled from some batches of Hout Bay lobsters, which were individually placed in an Eppendorf tube for storage at −25 °C.

Growth or moult increment (increase in lobster CL at moult) in each sampling area was determined from tagged- and recaptured animals, according to Goosen and Cockcroft (1995), during routine research cruises. Values were taken for the fishing seasons spanning from 2008−2009 to 2013−2014 so that they provide the context for the period of biological and biochemical sampling (described above).

Biochemical analyses of hepatopancreas samples were limited to Hout Bay lobsters to minimize cost and labor. These analyses were performed by the Council of Scientific and Industrial Research (CSIR) of South Africa as follows: After removal from the freezer, hepatopancreas weight was determined, the sample defrosted and homogenized using a Ultraturrax blender (IKA, Germany). Subsequently, moisture content (by heating for 24 h at 104 °C), crude protein (LECO protein analyzer, measurement of nitrogen by standard DUMAS; % nitrogen was converted to % protein using the factor 6.25) and total lipid content (Bligh and Dyer, 1959) were determined from aliquots. Ash and carbohydrate contents were determined by difference (% Carbohydrates and Ash = 100 % - sum of (% lipids + % moisture + % protein).

Somatic indices, i.e. hepatosomatic index (HSI) and abdominal muscle index (TI) were calculated according to equations:

$$HSI = \frac{Hepatopancreas\ \ wet\ \ weight}{Total\ \ wet\ \ weight} \times 100\% \quad (1)$$

$$TI = \frac{Tail\ \ wet\ \ weight}{Total\ \ wet\ \ weight} \times 100\% \quad (2)$$

The stage of individual Hout Bay lobsters in the moult cycle was determined via microscopic analysis of setagenic features of pleopods as outlined in Marco (2012) for adult *J. lalandii*. Briefly, the distal section of the pleopods were floated on a cavity slide, covered with a cover slide and examined with a compound eclipse Ni microscope (Nikon, Japan). The moult stages of *Jasus lalandii* are divided into stages AB (post--moult), C (intermoult), D_0 - D_{3-4} (pre-moult) (Marco, 2012). Substages of premoult stage D_1, such as D_1', D_1", were lumped together for simplicity and all moult stages were later coded as numerical values for analytical purposes (C = 1, D_0 = 2, D_1 = 2.5). This analysis was also limited to Hout Bay lobsters to minimize labor.

Statistical differences in biological and biochemical parameters and linear regression statistics were analyzed by one-way ANOVA followed by the appropriate parametric- or nonparametric post-hoc test (given in figure captions). All statistics were calculated by using Sigma Plot version 14.0. The significance level chosen throughout was $p < 0.05$.

3. Results

3.1. Growth increment

Except in the 2009−2010 season, when it was almost equal, mean moult increments (Table 1) measured in Hout Bay were substantially larger than those in the Olifantsbos area. The lowest value in Hout Bay of 2.5 mm in 2009−2010 was close to the highest value in Olifantsbos in all years analysed. Due to unavailability of sea time, there was no growth rate measurement in Hout Bay in the 2012−2013 season.

3.2. Biological data

Due to the targeted size class of lobsters, variation in average total

Table 1
Mean moult increments (mm) measured in the Hout Bay and Olifantsbos areas during 2008-2014 from tagged- and recaptured animals.

Fishing season	n	Hout Bay	n	Olifantsbos	% difference
2008−2009	309	2.2 ± 1.9	283	1.6 ± 1.4	27*
2009−2010	383	2.5 ± 1.8	510	2.3 ± 1.5	7
2010−2011	34	2.7 ± 1.2	596	1.6 ± 1.3	41*
2011−2012	39	3.4 ± 1.9	37	1.5 ± 1.1	55*
2012−2013	n.a.	2.8#	67	1.8 ± 1.8	34#
2013−2014	33	3.4 ± 1.1	447	2.0 ± 1.7	40*

Values are given as mean ± SD.
* Significantly different between areas in the same fishing season ($p < 0.05$, t-test).
# No value determined. Estimated from average differences in growth increment of all other fishing seasons. No tag returns due to unavailability of own ships and no fishing in the area by industry.

wet weight (w_T) during the research period was low (Table 2). Lobsters from the Hout Bay sampling site ranged from 188 to 243 g in mean total weight (CL 70–76 mm) whereas in Olifantsbos total weight averaged 196–227 g (CL 71–75 mm).

Mean tail weight w_A ranged from 64 g in February 2011 to 80 g in December 2010 in Hout Bay and from 64 g in July 2010 to 74 g in February 2012 in Olifantsbos (Table 2). Average TI ranged from 32 % of w_T in July 2010 and November 2011 to 34 % in several months of each year in Hout Bay (Table 2, Fig. 2A). In Olifantsbos it ranged from 32 % to 34 %, each in several months of each year (Table 2, Fig. 3A). There was no clear seasonal trend in either area. The mean tail weight : carapace length (w_A/CL) ratio in Hout Bay ranged from 0.9 to 1.1 throughout the sampling period (Table 2, Fig. 2B), although values were higher in each summer. Similarly, in Olifantsbos the range was 0.9–1.0 (rounded, Table 2, Fig. 3B). In general, the w_A/CL ratio is lower in Olifantsbos and never reached a value of 1. There was no trend in the Olifantsbos data.

In both areas, the mean hepatopancreas wet weight (w_H) was highest over the austral summer months after which it declined to reach a minimum in autumn/early winter (Table 2). In Hout Bay, it fluctuated between 8.6 g in October 2010 to 12.3 g in December 2010. Similarly, in Olifantsbos, w_H ranged from 9.0 g in November 2011 and 11.7 g in February 2012.

In Hout Bay, mean Hepatosomatic Index (HSI) ranged from 4.3 in October 2010 to 5.4 % of w_T in January 2011. It was generally high around December to April each year and lowest several months later (Table 2, Fig. 2C). Lowest values occurred just before and after moult before increasing to peak values between January and May. In Olifantsbos, the general trend was similar with the lowest value of 4.4 % measured in October 2010 and the highest of 5.3 % in May 2011 (Table 2, Fig. 3C).

3.3. Moult cycle

The stage in the moult cycle was determined from some batches (months) of lobsters in the Hout Bay area. All analyzed animals were either in intermoult stage C and premoult stages D_0 and D_1. (Fig. 4A). Stage D_1 consists of three sub-stages. For simplicity and given the small sample size, D_1 was not further divided and all sub-stages are summarized as D_1. Lobsters in stage C were present in all months sampled and numbers ranged from 4 to 26 with the highest number in March 2010 (26, 87 %) while the lowest was found in October 2011 (4, 16 %). It was also the dominant moult stage in animals caught in January (15, 60 %) and February (16, 64 %) 2011. The number of lobsters in stage D_0 ranged from 4 to 16 (16–64 %) and was present throughout each months' samples. The share of the stage was only low in March (4, 13 %) and July 2010 (9, 30 %) and February 2011 (9, 36 %); in all other months, it reached 40–64 %. The number of lobsters in stage D_1 ranged from 1 to 11 (4–37 %) and was never a dominant stage in any of the months sampled. D_1 was only observed in 4 of the 10 months sampled. The highest share was recorded in July 2010 (11, 37 %) and October 2011 (7, 28 %).

To achieve a clearer trend by possibly reducing yearly variation,

Table 2
Biological data of male *J. lalandi* caught at two sites from March 2010 to March 2012.

Date	n	CL mm	w_T g	w_A g	w_H g	TI % w_T	w_A/CL	HSI % w_T	Moult Stage numerical
Hout Bay									
Mar 2010	30	75 ± 3	228 ± 29	75 ± 8	11.3 ± 2.2	33 ± 2	1.0 ± 0.1	4.9 ± 0.6	1.1 ± 0.3
Apr 2010	30	76 ± 3	243 ± 31	79 ± 10	11.9 ± 2.0	33 ± 2	1.0 ± 0.1	4.9 ± 0.6	n.d.
May 2010	30	73 ± 2	210 ± 17	69 ± 5	10.9 ± 1.4	33 ± 1	0.9 ± 0.1	5.2 ± 0.6	n.d.
Jul 2010	30	72 ± 2	201 ± 19	65 ± 6	8.8 ± 1.1	32 ± 2	0.9 ± 0.1	4.4 ± 0.5	1.8 ± 0.7
Oct 2010	24	72 ± 2	199 ± 23	68 ± 7	8.6 ± 1.4	34 ± 2	0.9 ± 0.1	4.3 ± 0.6	1.7 ± 0.6
Nov 2010	25	73 ± 2	216 ± 19	72 ± 6	9.5 ± 1.9	34 ± 2	1.0 ± 0.1	4.4 ± 0.7	1.8 ± 0.5
Dec 2010	25	75 ± 4	237 ± 39	80 ± 13	12.3 ± 2.5	34 ± 2	1.1 ± 0.1	5.2 ± 0.6	1.6 ± 0.5
Jan 2011	25	73 ± 3	215 ± 23	73 ± 7	11.7 ± 1.3	34 ± 2	1.0 ± 0.1	5.4 ± 0.5	1.4 ± 0.5
Feb 2011	25	70 ± 4	188 ± 32	64 ± 10	9.9 ± 2.5	34 ± 2	0.9 ± 0.1	5.2 ± 0.7	1.3 ± 0.5
Apr 2011	25	71 ± 3	199 ± 23	68 ± 7	9.7 ± 1.7	34 ± 2	1.0 ± 0.1	4.8 ± 0.5	n.d.
Jul 2011	25	73 ± 2	210 ± 19	69 ± 6	10.3 ± 1.7	33 ± 2	1.0 ± 0.1	4.3 ± 0.8	1.2 ± 0.4
Oct 2011	25	72 ± 3	203 ± 21	69 ± 6	8.6 ± 1.7	34 ± 2	1.0 ± 0.1	4.3 ± 1.1	1.9 ± 0.5
Nov 2011	25	73 ± 2	206 ± 16	65 ± 4	9.5 ± 1.2	32 ± 1	0.9 ± 0.0	4.6 ± 0.6	n.d.
Dec 2011	23	75 ± 7	241 ± 70	79 ± 22	11.2 ± 2.3	33 ± 1	1.0 ± 0.2	4.8 ± 0.9	n.d.
Jan 2012	25	75 ± 3	228 ± 29	78 ± 11	12.0 ± 2.1	34 ± 2	1.0 ± 0.1	5.2 ± 0.5	n.d.
Feb 2012	25	75 ± 3	223 ± 23	73 ± 6	11.2 ± 1.5	33 ± 2	1.0 ± 0.1	5.0 ± 0.6	n.d.
Mar 2012	25	75 ± 3	228 ± 29	78 ± 11	12.2 ± 2.2	34 ± 2	1.0 ± 0.1	5.3 ± 0.5	1.7 ± 0.5
Olifantsbos									
Mar 2010	30	72 ± 2	207 ± 24	71 ± 8	10.1 ± 2.1	34 ± 1	1.0 ± 0.1	4.8 ± 0.8	n.d.
Apr 2010	30	71 ± 2	196 ± 23	66 ± 7	9.2 ± 1.7	34 ± 2	0.9 ± 0.1	4.8 ± 0.7	n.d.
May 2010	30	74 ± 2	219 ± 20	73 ± 5	11.1 ± 1.7	33 ± 1	1.0 ± ± 0.0	5.0 ± 0.5	n.d.
Jul 2010	30	71 ± 2	199 ± 19	64 ± 6	9.6 ± 1.0	32 ± 1	0.9 ± 0.1	4.5 ± 0.5	n.d.
Oct 2010	30	74 ± 2	215 ± 20	72 ± 6	9.4 ± 1.7	33 ± 2	1.0 ± 0.1	4.4 ± 0.6	n.d.
Nov 2010	30	72 ± 2	214 ± 21	71 ± 6	9.8 ± 1.8	33 ± 2	1.0 ± 0.1	4.6 ± 0.8	n.d.
Dec 2010	30	72 ± 1	203 ± 15	65 ± 5	9.3 ± 1.3	32 ± 2	0.9 ± 0.1	4.9 ± 0.6	n.d.
Jan 2011	30	71 ± 2	213 ± 17	68 ± 7	10.1 ± 1.8	32 ± 2	0.9 ± 0.1	5.1 ± 0.6	n.d.
Feb 2011	30	72 ± 2	198 ± 23	66 ± 7	9.9 ± 2.1	34 ± 2	0.9 ± 0.1	5.1 ± 0.6	n.d.
Apr 2011	30	72 ± 3	208 ± 22	66 ± 7	10.2 ± 1.8	32 ± 2	0.9 ± 0.1	4.9 ± 0.7	n.d.
May 2011	30	72 ± 2	205 ± 14	67 ± 5	11.6 ± 1.5	33 ± 2	0.9 ± 0.1	5.3 ± 0.5	n.d.
Jul 2011	30	72 ± 2	206 ± 20	67 ± 6	11.3 ± 2.2	33 ± 2	0.9 ± 0.1	4.8 ± 0.6	n.d.
Oct 2011	30	72 ± 2	203 ± 19	69 ± 7	10.3 ± 1.5	34 ± 2	1.0 ± 0.1	4.7 ± 0.5	n.d.
Nov 2011	30	72 ± 2	197 ± 21	67 ± 7	9.0 ± 1.3	34 ± 2	0.9 ± 0.1	4.6 ± 0.6	n.d.
Dec 2011	30	72 ± 2	204 ± 17	66 ± 5	10.4 ± 1.6	32 ± 1	0.9 ± 0.0	4.9 ± 0.6	n.d.
Jan 2012	30	73 ± 2	204 ± 16	65 ± 5	11.0 ± 2.3	32 ± 2	0.9 ± 0.0	5.0 ± 1.0	n.d.
Feb 2012	30	75 ± 2	227 ± 21	74 ± 5	11.7 ± 1.8	33 ± 1	1.0 ± 0.0	5.2 ± 0.7	n.d.
Mar 2012	30	73 ± 2	210 ± 21	67 ± 6	10.4 ± 2.3	32 ± 2	0.9 ± 0.1	5.0 ± 1.0	n.d.

Values are given as mean ± SD. n.d. = not determined. Abbreviations: CL = carapace length, w_T = total wet weight, w_A = tail wet weight and w_H = hepatopancreas wet weight, TI = Tail Index, HSI = Hepatosomatic Index.

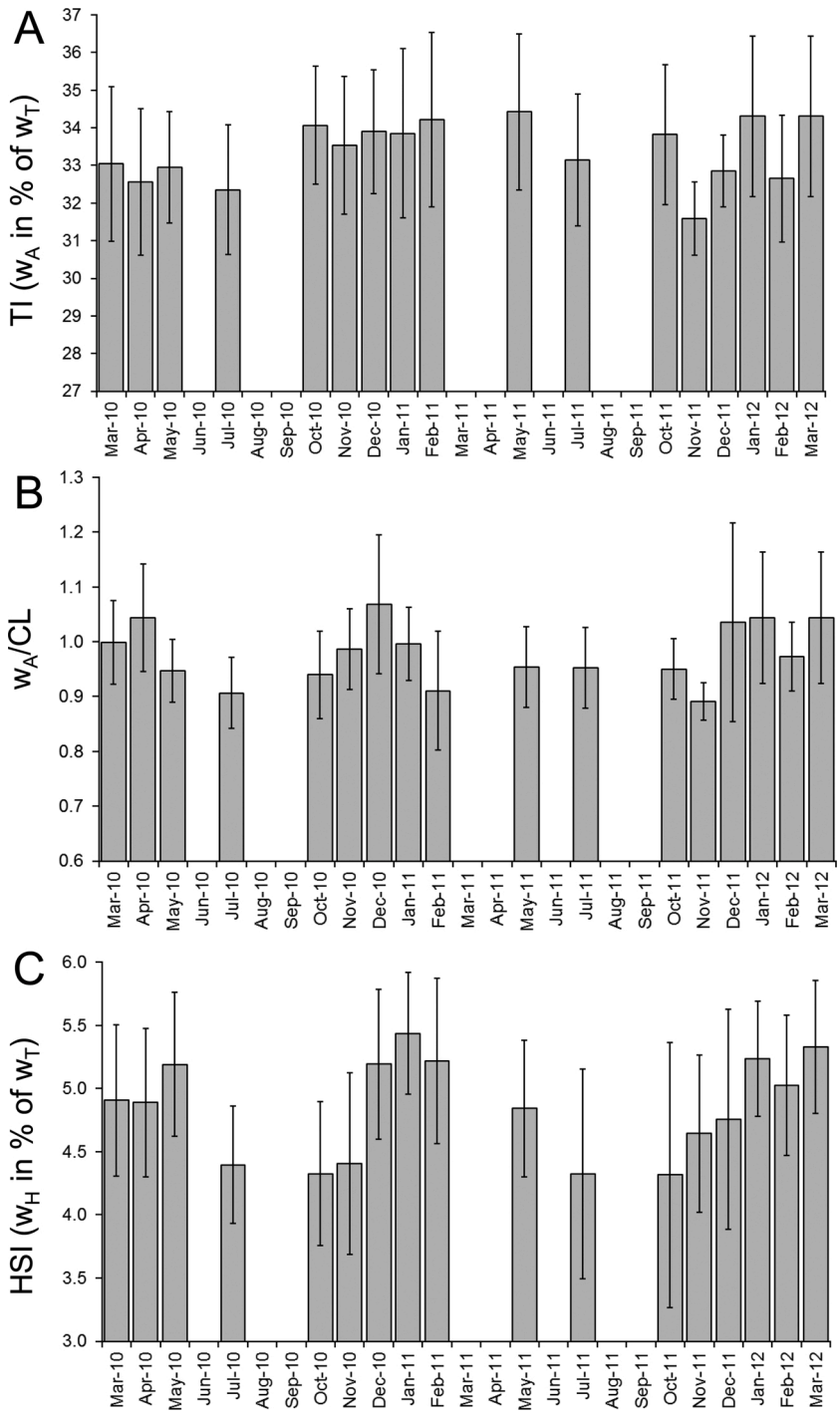

Fig. 2. The course of biological parameters from male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. A) Tail Index (TI), B) ratio of tail weight and Carapace length (w_A/CL) and C) Hepatosomatic Index (HSI). Values are means ± S.D., n = 23-30. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

moult stage numbers were accumulated, where possible, for the same months of the whole sampling period (Fig. 4B). These accumulated results show that lobsters caught throughout the year are mainly in stages C and D_0 (80–100 %) and that only before the moulting season, animals in D_1 occurred in relevant numbers (8–20 %). Early in the year (February – April), most lobsters (60–64 %) were in intermoult stage. Thereafter, this percentage declines to below 30 % into November and increases again towards end of the year (44 %). The share of lobsters in stage D_0 increased from 34 to 40 % early in the year to a maximum of 64 % in November. In December, their share is lower again at 56 %. To present the above data in a simple, continuous trend, moult stages were coded and converted to numerical values (Table 2, Fig. 4C). Early in the moult cycle (stage C), values would be low and increase as the cycle progresses. The lowest value of 1.1 was recorded in March 2010 and the highest of 1.9 in October 2011. Lowest values were usually recorded early each year and highest in the second half of the year.

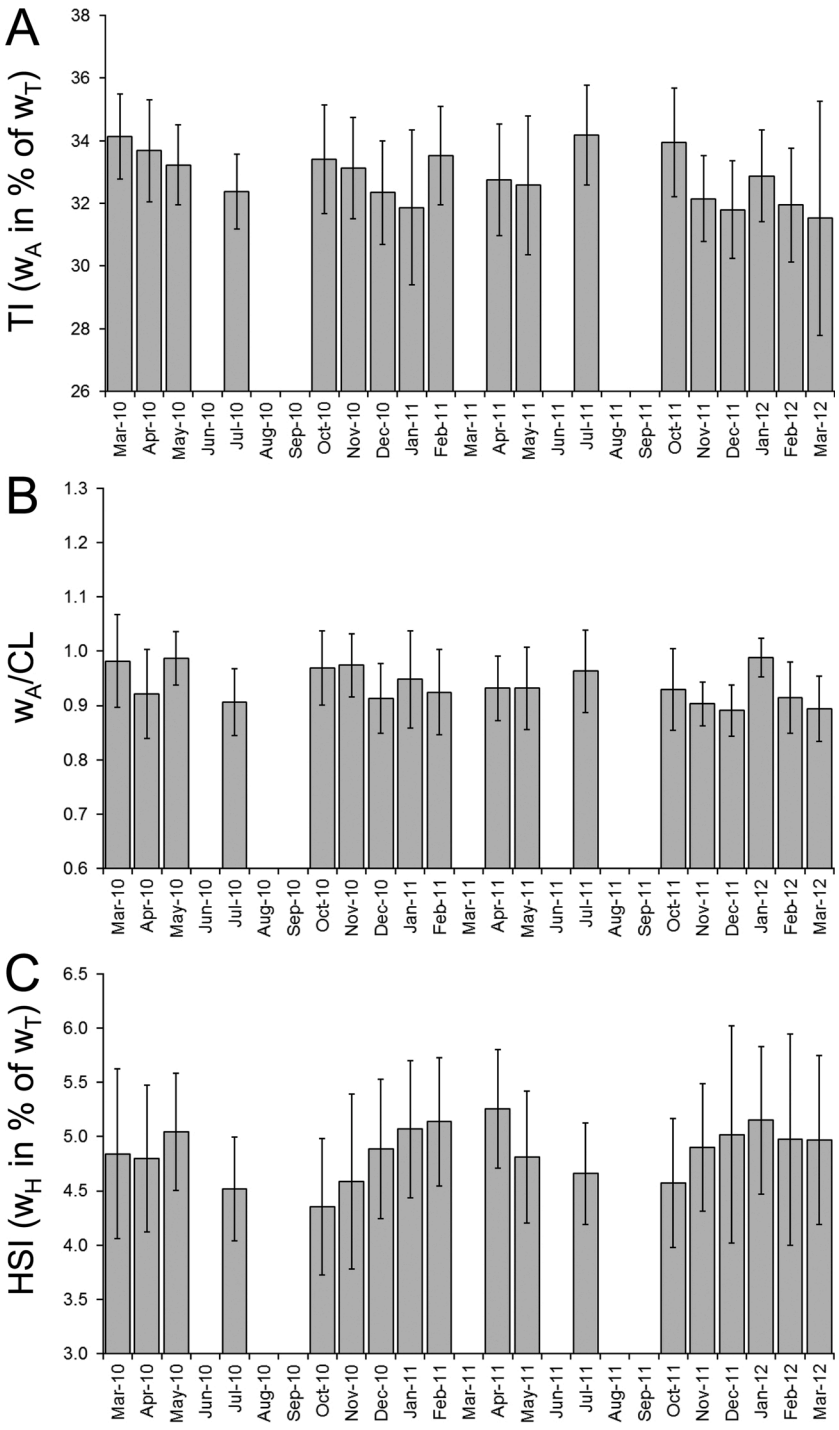

Fig. 3. The course of biological parameters from male *J. lalandii* caught at the Olifantsbos sampling site during the period from March 2010 to March 2012. A) TI, B) w_T/CL and C) HSI. Values are means ± S.D., n = 30. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight.

3.4. Hepatopancreas biochemistry

3.4.1. Moisture content

The mean moisture content of the hepatopancreas is inverse to w_D (Table 3) and is therefore not explicitly reported here. It serves, however, as reference value for the content of other compounds and is used to determine the actual grams of lipid and protein in the hepatopancreas on a dry weight basis. Moisture content ranged from 63.7 % in January 2012 to 75.5 % in October 2010. Highest moisture contents were recorded in summer, i.e. before moulting after which they declined until winter (Table 3; Fig. 5A).

3.4.2. Lipid content

Lipid content (per w_D) was lowest in October to December, followed by an increase to peak values during March to July and a subsequent decline (Table 3; Fig. 5B). Peak values were in the range of 48–52 % and lowest values in the range of 27–36 %.

3.4.3. Protein content

Protein content (per w_D) follows a similar trend as moisture content, both of which are inverse to that of lipid content (Fig. 5). Lowest values of approximately 40 % were recorded in autumn whereas peak values of about 50–55 % occurred from October to December.

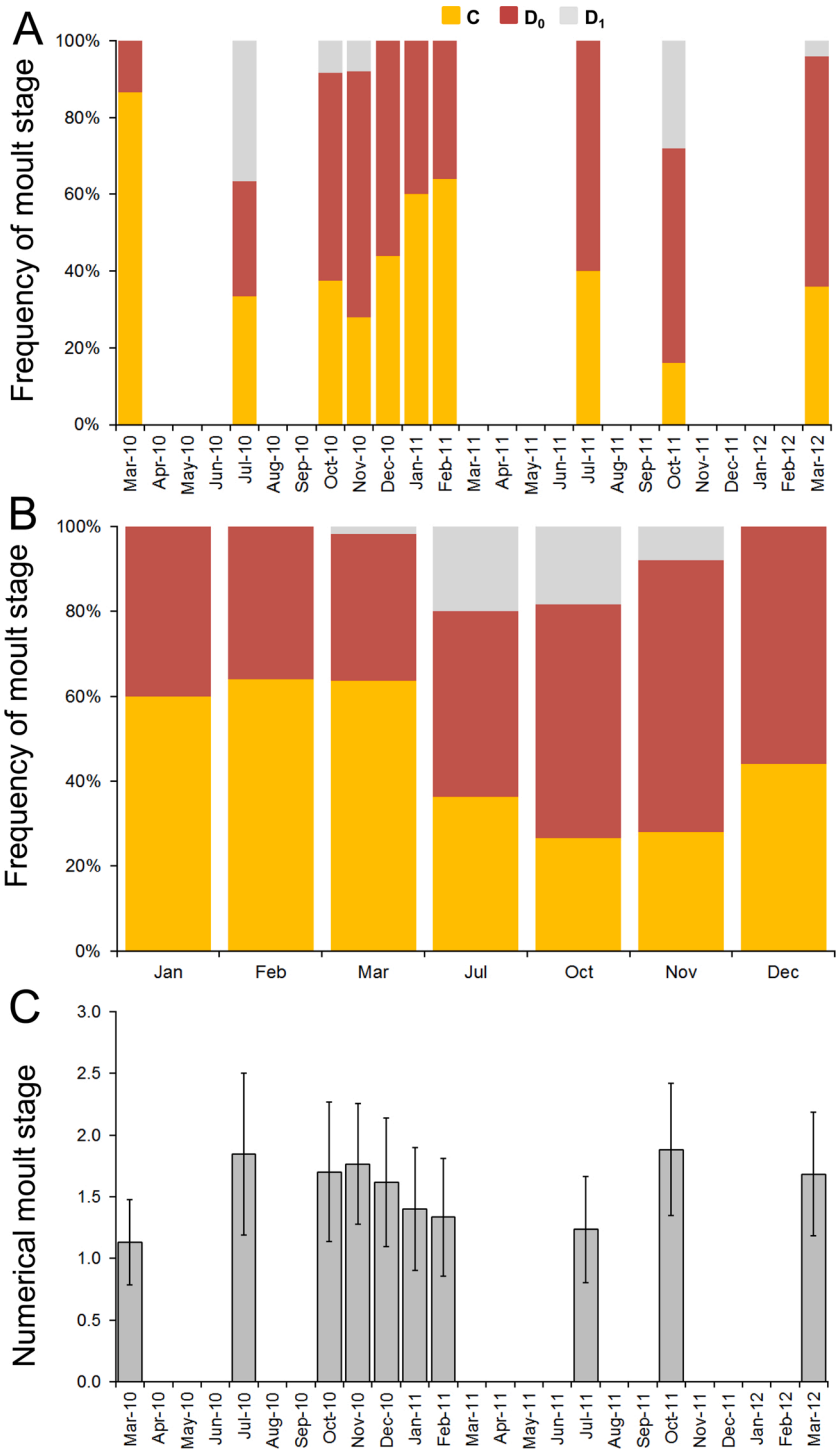

Fig. 4. Moult stage of male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during select months from March 2010 to March 2012. A) relative frequency of moult stage distribution, B) accumulated moult stage frequency for same months of all sampled years and C) numerical course of stage in moult cycle throughout the months of the sampling period (coded C = 1, D_0 = 2, D_1 = 2.5). In C, values are means ± S.D., n = 24-30.

3.4.4. Carbohydrate and ash content

The course of these compounds (per w_D) seemed to follow the trend of total proteins but much less pronounced (Fig. 5B). Lowest values of approximately 8–12 % occurred May to July whereas highest values of about 15–18 % were recorded in late spring/early summer (Table 3 Fig. 5B).

3.5. Potential indicators

The biological indicators HSI and TI were analyzed per moult stage for the Hout Bay sampling site, for individual animals whose moult stage had been determined (see above). For this, values of the same stage from the entire sampling period were pooled, i.e. all intermoult stage C (n = 117) and premoult stages D_0 (n = 117) and D_1 (n = 25). Their respective HSI and TI were then compared (Fig. 6). HSI values were very similar in all three moult stages ~5 % and only D_1 was ~8 % lower than the two

Table 3
Biochemical composition of hepatopancreas from male *J. lalandi* caught at the Hout Bay sampling site ("Die Knol") from March 2010 to March 2012.

Date	n	moisture % w_W	w_D % w_W	Total Lipids % w_D	Proteins % w_D	Carbohydrates and ash % w_D
Mar 2010	30	67.7 ± 5.2	32.3 ± 5.2	41.8 ± 10.5	43.9 ± 7.6	14.3 ± 5.3
Apr 2010	30	67.6 ± 5.2	32.4 ± 5.2	41.4 ± 9.9	43.9 ± 7.8	14.7 ± 5.0
May 2010	30	68.1 ± 5.2	31.9 ± 5.2	44.0 ± 10.4	41.9 ± 6.4	14.1 ± 5.9
Jul 2010	30	69.0 ± 5.4	31.0 ± 5.4	48.9 ± 10.4	39.9 ± 6.6	11.2 ± 6.6
Oct 2010	24	75.5 ± 6.3	24.5 ± 6.3	33.2 ± 6.5	50.3 ± 4.0	16.5 ± 7.0
Nov 2010	25	73.2 ± 7.0	26.8 ± 7.0	36.4 ± 11.0	51.9 ± 7.2	11.6 ± 5.3
Dec 2010	25	73.3 ± 4.5	26.7 ± 4.5	35.3 ± 14.6	49.9 ± 8.2	14.8 ± 9.3
Jan 2011	25	70.0 ± 8.1	30.0 ± 8.1	37.3 ± 11.4	47.0 ± 5.2	15.7 ± 8.1
Feb 2011	25	66.0 ± 4.2	34.0 ± 4.2	41.0 ± 13.7	44.9 ± 7.7	14.1 ± 6.6
May 2011	25	67.1 ± 4.3	32.9 ± 4.3	48.5 ± 10.8	41.9 ± 6.9	9.6 ± 5.0
Jul 2011	25	68.0 ± 2.4	32.0 ± 2.4	51.6 ± 14.5	39.9 ± 9.1	8.5 ± 6.9
Oct 2011	25	71.7 ± 4.5	28.3 ± 4.5	34.0 ± 8.8	50.0 ± 4.7	16.0 ± 7.0
Nov 2011	25	69.0 ± 5.8	31.0 ± 5.8	26.8 ± 6.3	55.1 ± 6.2	18.1 ± 6.9
Dec 2011	25	65.6 ± 6.0	34.4 ± 6.0	35.9 ± 10.8	50.0 ± 6.6	14.2 ± 8.1
Jan 2012	25	63.7 ± 5.9	36.3 ± 5.9	40.1 ± 8.0	46.0 ± 6.3	14.0 ± 9.3
Feb 2012	25	66.6 ± 3.1	33.4 ± 3.1	41.3 ± 9.2	44.9 ± 5.5	13.8 ± 7.0
Mar 2012	25	65.2 ± 4.0	34.8 ± 4.0	43.2 ± 10.9	42.3 ± 5.4	14.5 ± 6.8

Values are given as mean ± SD. n.d. = not determined. Abbreviations: w_W = wet weight, w_D = dry weight.

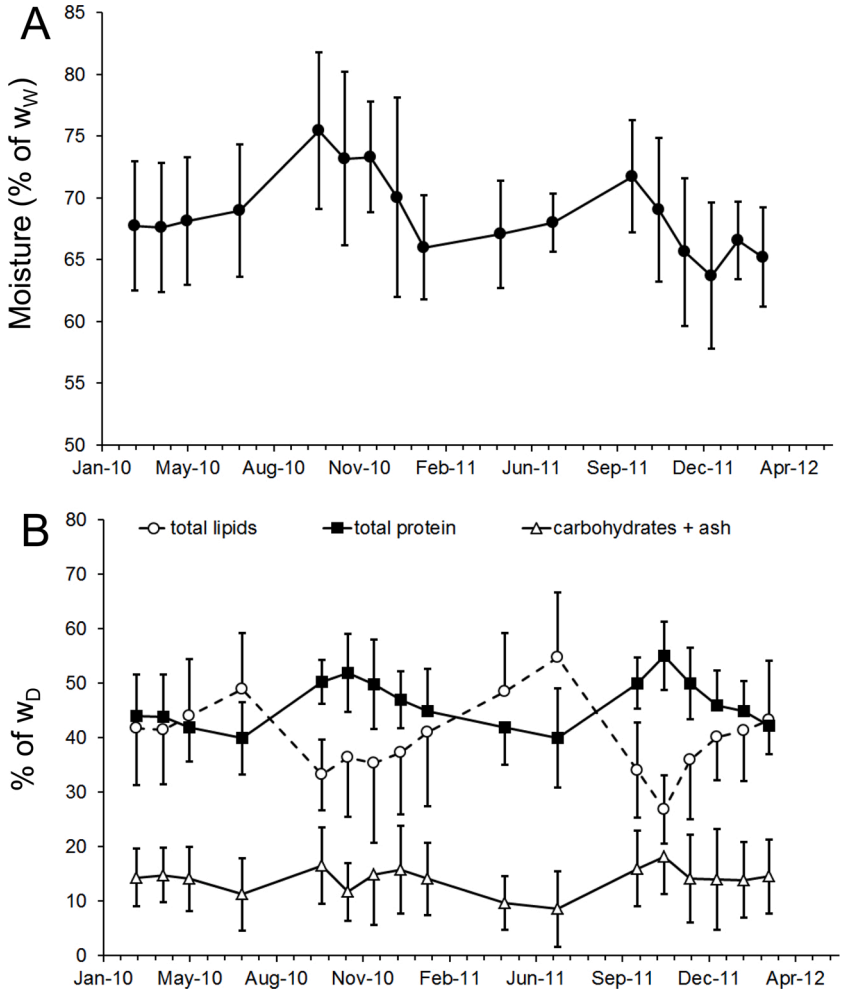

Fig. 5. The course of the biochemical composition of hepatopancreas samples from male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. A) Moisture content, B) lipid-, protein- and carbohydrate and ash contents. Values are means ± S.D., n = 24-30. Abbreviations: w_W = wet weight, w_D = dry weight.

other stages (not significant) at 4.6 % (Fig. 6A). TI was highest (33.7 %) in intermoult (stage C) and declined slightly during premoult stages D_0 (33.5 %) and D_1 (32.9 %, not significant; Fig. 6B). There is a declining trend in TI from stage C to stage D_1 from 33.7–32.9% (-2.4 %) although these differences were not significant. Hepatopancreas lipid content was highest in stage C (13.2 %) and at a 11 % lower level (~11.7 % of w_D) at stages D_0 and D_1, respectively (Fig. 6C). The differences were not significant. Protein content was at a very similar level of approximately 13

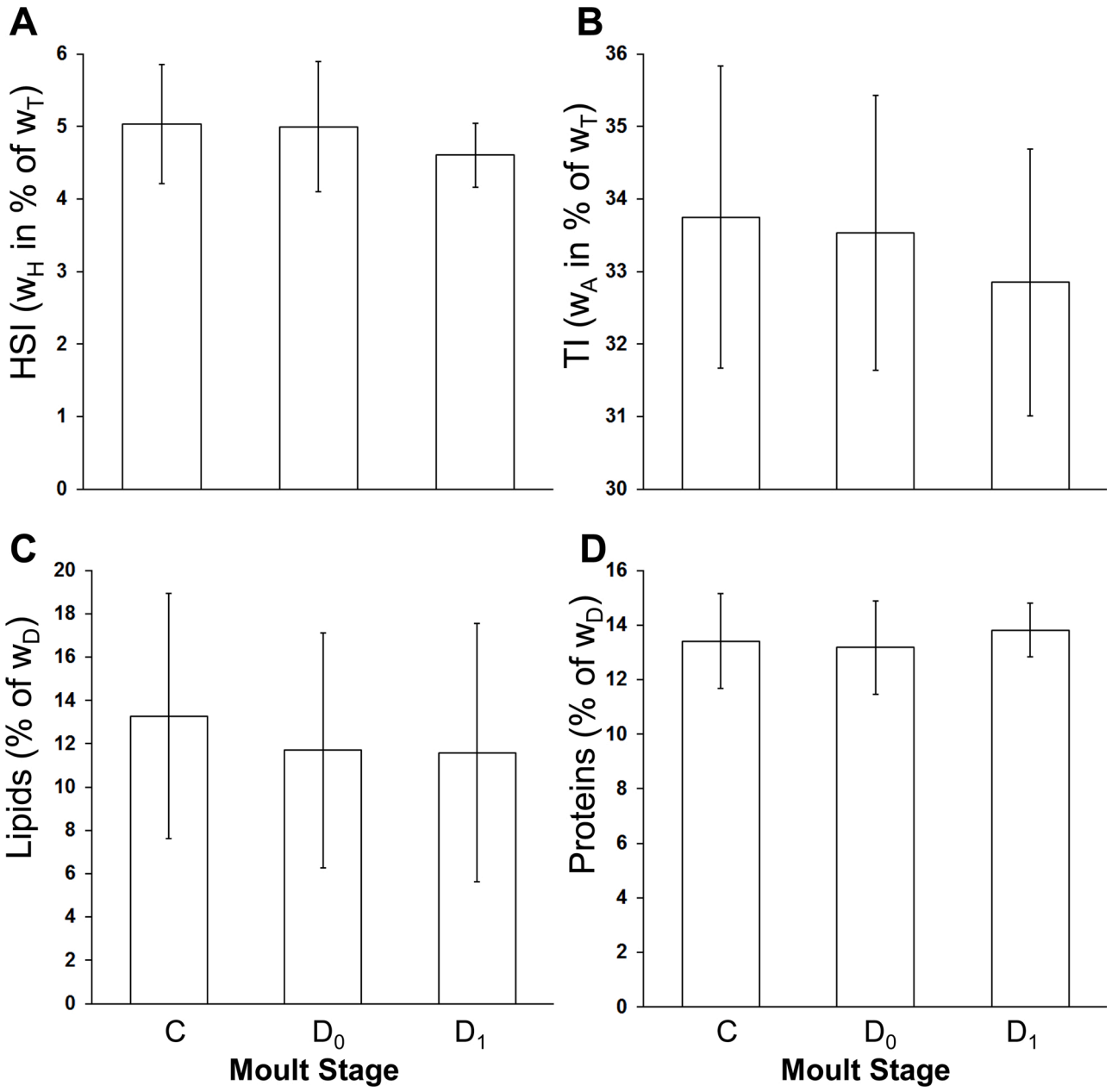

Fig. 6. Levels of A) HSI, B) TI, C) hepatopancreas lipid content and D) hepatopancreas protein concentration during the respective moult stage. Analysis was carried out on lobsters from the Hout Bay sampling site only. No significant differences ($p < 0.05$) between moult stages were observed (one-way ANOVA). Values are means ± S.D., n = 117 (stage C), n = 117 (stage D_0), n = 23 (stage D_1). Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_D = dry weight.

% at each stage (Fig. 6D).

Based on maximum HSI, "peak accumulation" months were identified (Cockcroft, 1997). From this period of each year (2010: Mar, Apr, May; 2011: Jan, Feb, Apr; 2012: Jan, Feb, Mar), certain biological and biochemical parameters were accumulated and analysed together. Whereas HSI was in a narrow range of about 5.0–5.5 % in Hout Bay, values for 2011 and 2012 were significantly higher than that for 2010 (Fig. 7A). In Olifantsbos, values ranged from 4.9 to 5.2 % and the value for 2012 was significantly higher than in the preceding years (Fig. 7E). TI was similar in each peak period in Hout Bay at 33–34 % (Fig. 7B). Despite this narrow range, values for 2011 and 2012 were significantly higher than in 2010. TI declined significantly from 34 to 30 % in Olifantsbos from 2010 to 2012 (Fig. 7F). Lipid content in Hout Bay was highest in 2010 (17.2 %) and at a level of about 12 % in both of the following years (Fig. 7C). Protein was lower at around 13 % in both 2010 and 2011 than the 15.3 % in 2012 (Fig. 7D).

Analysis of interrelationships of hepatopancreas parameters of all animals sampled at the Hout Bay site revealed that there is a decrease in moisture content as hepatopancreas size increases (Fig. 8A). Lipid- and protein concentrations showed increasing and decreasing trends, respectively, as hepatopancreas size increases, although with shallow slopes (Fig. 8B,C). These inverse trends are also visible in the significant correlation of both compounds in Fig. 8D.

Lobsters were, with few exceptions, in a narrow size range and hence comparable. Absolute lipid weight per hepatopancreas was therefore used as a factor to estimate the total amount of lipids available for growth. First, a seasonal course was constructed for this parameter (wet weight lipid concentration multiplied by hepatopancreas wet weight): Highest values occurred at the beginning of each calendar year (1.4–1.9 g) and lowest (0.8 – 0.9 g) in austral spring (Fig. 9). The highest value in

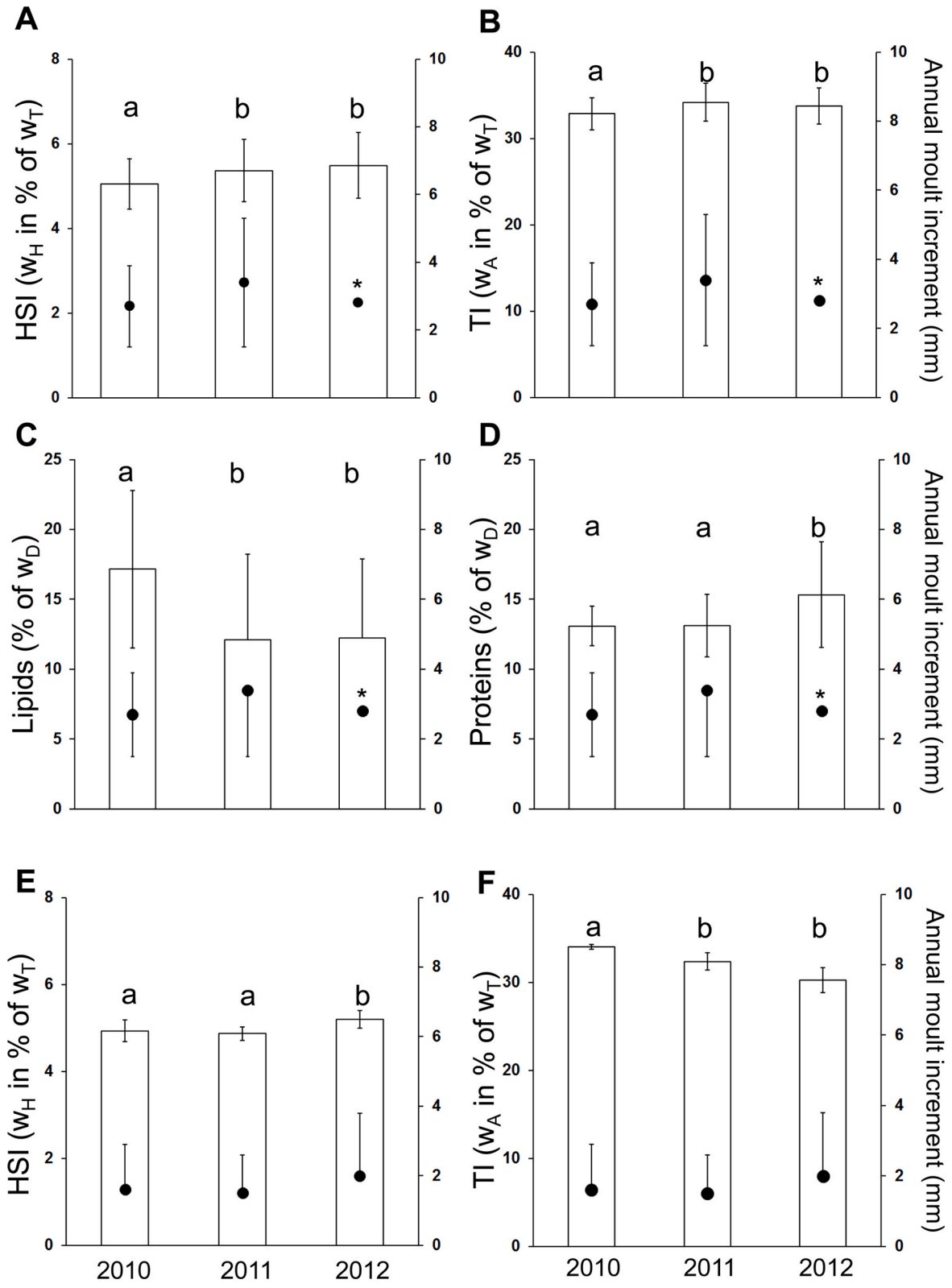

Fig. 7. Biological and biochemical data (bars, left y-axis) from male *J. lalandii* caught at both sampling sites during the respective annual peak accumulation periods from March 2010 to March 2012. A) HSI, B) TI, C) lipid content and D) protein content from the Hout Bay sampling site. E) HSI and F) TI from the Olifantsbos sampling site. For information, respective annual growth rates are given as full circles (y-axis). Note, no annual growth rate is available for the Hout Bay sampling site for 2012 due to unavailability of ships. *Estimated, see Table 1. Different lower case letters indicate a significant difference ($p < 0.05$) between years (one-way ANOVA followed by Kruskal-Wallis Analysis). Values are means ± S.D., n = 55-90. Abbreviations: w_T = total wet weight, w_A = tail weight and w_H = hepatopancreas wet weight, w_D = dry weight.

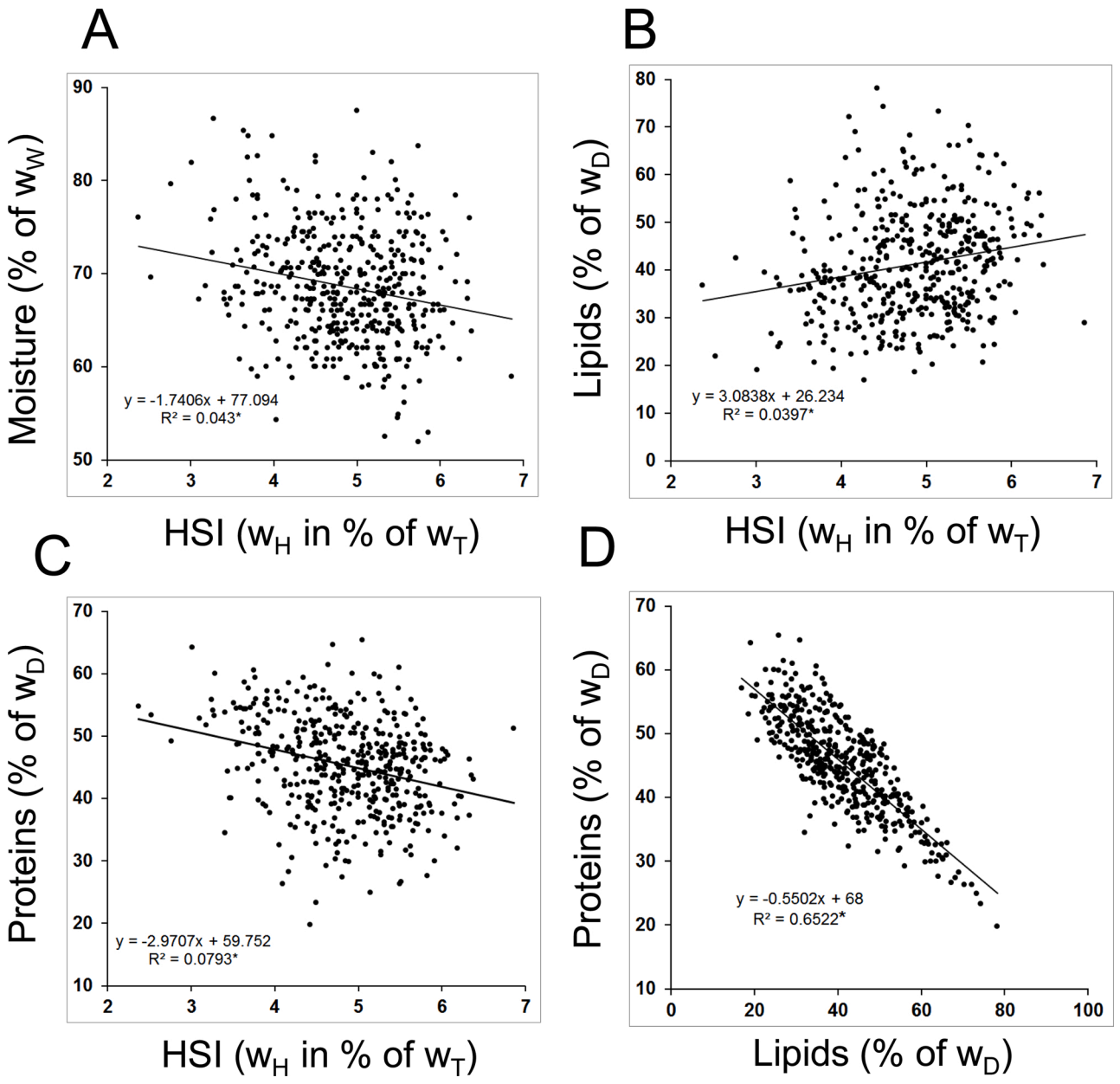

Fig. 8. Interrelationships of hepatopancreas A) relative weight (HSI) and moisture content, B) HSI and lipid concentration, C) HSI and protein concentration and D) lipid concentration and protein concentration from all WCRL analysed from the Hout Bay sampling site (n = 442). *significant correlation ($p < 0.05$) of linear regression (ANOVA). Abbreviations: w_W = wet weight, w_D = dry weight, w_T = total wet weight, w_H = hepatopancreas wet weight.

2011 was observed in July (1.7 g) whereas it was highest (1.6 g) in March in 2010 (Fig. 9, Table 4). Although lobsters caught in July 2011 where slightly larger, the amount of lipid per hepatopancreas was 24 % higher, owing to both the larger hepatopancreas weight and the higher lipid concentration. Although lipid weight per hepatopancreas only peaked in July in 2011, lipid concentration in the hepatopancreas peaked in July of both years (Table 3, Fig. 5B). July was also the last sampling point before moult. Data for July 2010 and 2011 were therefore further analysed for their potential to predict growth in the subsequent moult. In 2010, lipid content was higher both per w_D (+12 %) and w_W (+ 9 %) in 2011 than in 2010. In addition, lipid per hepatopancreas was 24 % higher in 2011, whereas w_T as well as HSI were only slightly higher (Table 4).

4. Discussion

The present research has confirmed some previous findings: Long-standing growth trends and differences between the two sampling sites (Goosen and Cockcroft, 1995; Cockcroft, 1997), that triggered previous studies, persist. Annual growth increment is still consistently higher by more than 1 mm (0.2–1.9 mm) at the Hout Bay sampling site than in Olifantsbos (Table 1) over the years evaluated in the present study. The causes of the difference as well as strong inter-annual growth variations are not well understood (Cockcroft, 1997). It was assumed that food availability is the reason for geographic differences but also for annual variations (Newman and Pollock, 1974; Pollock, 1982; Chandrapavan et al., 2009, 2010). Mean growth increment in the present study ranged from 2.2–3.4 mm in Hout Bay and 1.5–2.3 mm in Olifantsbos. This compares with 2.0–4.2 mm and 1.3–2.2 mm, respectively, of similar-sized lobsters in Cockcroft (1997). The latter research was

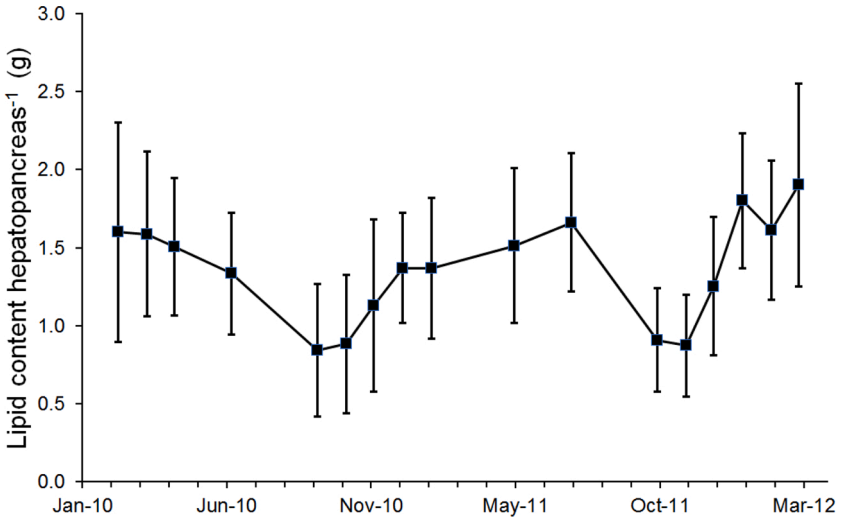

Fig. 9. The course of the lipid weight per hepatopancreas of male *J. lalandii* caught at the Hout Bay sampling site ("Knol") during the period from March 2010 to March 2012. Values are means ± S.D., n = 23-30.

Table 4
Annual differences in select biological and biochemical data from July 2010 and July 2011 from the Hout Bay fishing area.

	n	Growth increment mm	w_T g	W_H g	HSI % w_T	Lipid dry weight % w_D	Lipid wet weight % w_W	Lipid per hepatopancreas g
July 2010	30	2.7 ± 1.2	201 ± 19	8.8 ± 1.1	4.4 ± 0.4	48.9 ± 10.4	15.1 ± 4.2	1.3 ± 0.4
July 2011	25	3.4 ± 1.8	210 ± 19	10.1 ± 2.0	4.6 ± 0.5	51.6 ± 14.5	17.3 ± 3.5	1.7 ± 0.4
difference		26 %*	4 %	9 %	6 %*	12 %*	9 %*	24 %*

Values are given as mean ± SD. Abbreviations: w_T = total wet weight, w_W = wet weight. w_D = dry weight, w_H = hepatopancreas wet weight, HSI = Hepatosomatic Index.

* Significantly different between July 2010 and July 2011 ($p < 0.05$, t-test).

conducted in a period of low growth or even shrinkage along the South African West coast (Melville-Smith et al., 1995; Cockcroft and Goosen, 1995; Pollock et al., 1997). Values are still lower than in the past before the slow growth period (DEFF, 2020), indicating that factors for low growth generally persist. A difference in growth increment of 1 mm of a 200 g lobster seems relatively small. It results, however, in a body weight growth differential of approximately 2.8 g (~1.4 %) as calculated according to the conversion of Heydorn (1969b). It can therefore be concluded that (standardized 200 g) lobsters from Hout Bay grew by 2 g more in 2011 than in 2010. This differential growth seems to be area specific since in Olifantsbos, there was no difference between the two years (Table 1).

Moreover, seasonal trends in reserve accumulation in the hepatopancreas in the present study are like those found in Cockcroft (1997); an annual increase of HSI until mid to end summer at both sampling sites (Figs. 2,3) and an accumulation of lipids until end of autumn (Fig. 4). The lipid peak did not exactly coincide with the HSI peak and lags several months, results similar to Cockcroft (1997). This is, however, possibly a result of a less defined HSI peak compared with that of lipids in both studies. Lipid content was highest during late intermoult- to early premoult seasons and then decreased rapidly, reaching lowest values immediately before and after moulting. This may indicate their catabolism for energy but also as structural components in processes involved in moult, i.e., muscular- and exoskeleton growth, which start several months before moult. The lipid contents recorded here are at the level known for the palinurid hepatopancreas which can reach 70–75 % w_D in the late pre-moult stage and indicate its prominent role in lipid digestion and accumulation (Cockcroft, 1997; McLeod et al., 2004; Smith et al., 2004). Lipids are particularly relevant for biological processes such as growth (Smith et al., 2004; Perera and Simon, 2015). Furthermore, moisture content and relative protein content follow the same inverse relationship to lipids (Figs. 5,8D) as in the previous study (Cockcroft, 1997): when lipids peak, moisture and relative protein content are lowest (Fig. 5). Water seems to be replaced by the accumulating lipids and proteins as the hepatopancreas grows. Absolute protein levels (not shown) rise at a slower pace than lipids, causing it to decline in relative terms. Based on wet weight, protein levels peak a few months before lipids, similar to Cockcroft (1997). Trends in the tail muscle were less clear which is also similar to the previous study. The tail muscle constitutes a high portion of the total weight of a lobster (~34 % with exoskeleton) compared to that of the hepatopancreas of ~5 %. In addition, it is the main portion of muscle. Its size and composition may therefore be indicative of physiological condition. In the present study, relative weight of the abdomen (TI, measured as whole tail weight including exoskeleton) from lobsters of the two sites was in a similar range (29–34 %). Although the abdominal muscle is considered an energy reserve in most crustaceans, it did not appear to be utilized for energy provision here, at least not as judged by organ size. It was previously suggested that abdominal muscle reflects more long-term changes and responds less sensitively to environmental and physiological changes, despite its large relative size (Cockcroft, 1997).

In *J. lalandii* fisheries research, moult stage was previously, if at all, recorded on the basis of shell hardness and stages were subdivided into Hard, Hard Old, Soft New, and Hard New (Heydorn, 1969a). Since this is relatively subjective and needs some experience, the present study used setagenic analysis of pleopods. Results revealed that this method creates some challenges, too (see below). Analysis of moult stage composition of lobsters from the Hout Bay area revealed that there is a large variation and overlap of stages at almost all times of the season (Fig. 4A–C). For example, lobsters in stages C and D_0 were recorded in every month sampled. There may have been a margin of error when differentiating between stages C and D_0 in the present study. These two stages are

sometimes difficult to set apart, since no new setae are visible yet in stage D_0 (only from stage D_1"), the gap between old and new epidermis is still very narrow and, in addition, this gap is potentially obscured by pigmentation in adults (Marco, 2012). On the other hand, Marco revealed, based on haemolymph moult hormone titers, that adult *J. lalandii* stay longer in pre-moult stages, especially D_0, than previously estimated from morphological analysis (i.e. setagenic analysis of pleopods). Lobsters do not feed for 15–20 days when they are in stages D_3 to B or early C (Charmantier-Daures and Vernet, 2004; Marco, 2012), therefore, around moulting season, values for such non-feeding lobsters do not appear in the results as they cannot be caught by baited traps. This will, of course, increase the share of lobsters in all other (i.e. feeding) moult stages. As a result, an attempt to capture moulting process numerically is not very conclusive (Fig. 4D). In addition, there was no sampling in August and September in this investigation for logistical reasons. These months, when HSI reaches its minimum (Cockcroft, 1997), could have provided more detail. Nevertheless, moult data provided an additional tool in analyzing biological events during the course of the growth cycle.

Distinct variation of a parameter over the moult cycle or season was deemed essential for consideration as potential growth predictor (Cockcroft, 1997). Two data sets fulfil such seasonality: a) the course of the annual moult cycle and b) the accumulation of metabolic reserves for growth.

In order to test a), biological and biochemical parameters were analysed per moult stage. This, however, did not reveal a clear and useful trend as differences were small and not significant (Fig. 7). Reasons for these inconclusive results may be the low number of D_1 values ($n = 16–24$) compared to the other stages ($n = 103–119$). In addition, stage C is more heterogeneous than the premoult stages (see above), as it is the longest stage (about 130 days, Marco, 2012) and can even be divided into 4 sub-stages by moult hormone titre but not morphologically (Marco, 2012). The latter could not be done in the present study. Accumulation in intermoult takes place over a long period, starting at very low deposition levels. Lipids, for example, accumulate mainly in C_4 to early premoult and rapidly decline from D_2 (Charmantier-Daures and Vernet, 2004; Munian et al., 2020). Levels of accumulation will therefore be very different at the beginning of intermoult period, when accumulation of reserves begins, compared with its end when accumulation reaches its maximum. Unfortunately, an important gap in sampling occurred in August and September when metabolite accumulation is at its lowest. Determination of moult stages seems therefore not a helpful tool for analysis of moult cycle reserve accumulation.

For evaluation of b), the seasonality of "peak accumulation" of reserve levels per year (as described previously in Cockcroft, 1997) were analysed as well as the biochemical dynamics of the hepatopancreas. In terms of "peak accumulation", the present results do not support the usefulness of certain body parameters, i.e., hepatopancreas moisture content and lipid levels (as a % of wet weight) at previously identified peak accumulation months, for the prediction of growth in adult male *J. lalandii*. Although the trend regarding hepatopancreas lipid content was clear and seasonal, peak accumulation was not different between years and was not related to growth rates. The hepatopancreas is the central organ for growth in decapods. It serves, amongst other functions, uptake and storage of nutrients, synthesis and catabolism of storage metabolites and provision of material and energy for moult and growth and is the principal organ for lipid metabolism (Gibson and Barker, 1979). Relative size and metabolite content of hepatopancreas are therefore important condition indicators and qualified for potential growth predictors in the previous study (Cockcroft, 1997). Relative weight of hepatopancreas (HSI) from lobsters of the two sites (4.3–5.8 %) was in the same range as had previously been reported for palinurids (Cockcroft, 1997; McLeod et al., 2004) and decapods in general (Gibson and Barker, 1979; Whyte et al., 1986; Dall, 1981). There were no differences between the sites despite the lower growth in Olifantsbos. HSI peaked around December to April/May each season, indicating the buildup of reserves for growth and moulting. Peaks are even less sharp than in previous research and the subsequent drop was not as pronounced as in Cockcroft (1997). This was probably a result of the smaller, and maybe insufficient, sample size in the present study. It is perhaps also an indication that biological events have spread more widely and are not as closely synchronized anymore. Females in berry, for example, occurred historically until October (Heydorn, 1969a) but have most recently been found in numbers as late as December (van Zyl, personal communication). We speculated that this may be a result of climate change. Accumulated Hout Bay peak HSI values were lower in 2010 than in the two following years, corresponding with the 2010 and 2011 moult increments. The 2012 moult increment was also higher than in 2010 but was only estimated. The same is true for TI. The w_A/CL ratio showed some seasonality in Hout Bay samples where it peaked around each year end, i.e., shortly after moult (Fig. 2B), whereas no such seasonality was observed in Olifantsbos (Fig. 3B). Cockcroft (1997) had previously not found any seasonality in either of the two sampling sites. This ratio was therefore not considered further as a potential growth predictor. It is noteworthy that w_A/CL ratio is generally lower in Olifantsbos (Table 2) and may therefore be useful in expressing area differences.

In the precursor study, biochemical analysis of the hepatopancreas, moisture content, protein- and lipid concentrations showed the required seasonality. However, of the "peak" values, only lipid concentration correlated with growth. It was therefore described as a simple and robust predictor for adult male growth in *J. lalandii*, based on measurements conducted from 1991 to 1995 in which the lipid content correlated with moult increment of the same year (Cockcroft, 1997). It was therefore recommended for inclusion into resource management as an indicator of the current year's growth increment but has not been implemented into stock assessment procedures more than twenty years later. Here, no such correlation was found: Lipid concentration was substantially higher in 2010 than in the following years. However, at least compared with 2011, this did not correlate with a higher growth rate. In addition to less pronounced seasonal changes and drawn-out individual life cycle events (see above), this could be a result of gaps in sampling that occurred in the present study due to logistical problems in some years. Concluding from the present results, sampling without gaps is necessary during all peak months, presumably from December to May. The course of hepatopancreas protein content (per wet weight and dry weight) is also strongly seasonal, qualifying for consideration as potential indicator. Unfortunately, in 2012, when protein content was higher during peak months, the corresponding growth value was only estimated. Protein quantitation is less labor-intensive than that of lipids and can be automated to a large extent. Its use as an indicator would therefore be of advantage. Correlation of both relative lipid and protein contents with HSI are significant but have a relatively low slope (Fig. 8B,C). It may indicate that a potential indicator could be found in the HSI, if analyzed differently. This may require determining the difference between peak- and bottom values and also requiring sampling from July to September.

Data from July of both years are the last data collected before moult. In addition, lipid concentration is highest in July of each sampling year, indicating possible usefulness as a predictor for growth increment in the subsequent moult. July data were therefore analysed in more detail. To obtain a more meaningful value, the highly seasonal hepatopancreas size was included in this analysis although July is several months after the proposed peak accumulation in March to May when HSI reaches its peak. The total amount of lipid per organ (hepatopancreas) available for growth was obtained in this way. The calculated total amount of lipids per hepatopancreas showed a clear seasonal trend, a prerequisite for suitability as an indicator (Cockcroft, 1997). However, this curve also revealed a peak in July 2011 but not in July 2010. Despite this, comparison of July data from both years revealed differences that coincide with growth increment: HSI, lipid content and lipid per hepatopancreas are all significantly higher in 2011 than in 2010, which correlates with 26 % (0.7 mm) faster growth during subsequent moult in 2011 than in

2010 (Table 4). In 2010, lipid per hepatopancreas declined already before July, possibly indicating their use for other purposes and sub-optimal food availability and hence resulting in a slower growth rate. In July 2011, hepatopancreas protein concentration is lower than in July 2010 (Fig. 5). As described above, it is inverse to lipid concentration and also displays a strong seasonal trend. It may in future be used for a growth predictor, too.

5. Conclusion

The present study confirms seasonal accumulation trends of metabolic reserves in male *J. lalandii* from a fast growth and a slow growth area. However, previously reported potential indicators for annual male growth were not robust enough here, possibly due to different seasonal reserve accumulation. Moreover, an attempt to use moult stage to identify a growth predictor failed. Instead, various lipid data from the immediate pre-moult period (July sampling point in the present study) appeared as more promising growth predictors. More research is required to confirm this finding and to add a quantitative component. For such research, sampling for "peak accumulation" should ideally span from December to May each season whereas sampling for lipid indicators should take place from July to September.

Author's contribution

A.M. collected field data, guided biochemical analysis, conducted data analyses, prepared the first manuscript and implemented corrections and improvements. L.A. and A.C. conceptualized the study, guided the project and preparation of manuscript. L.C.H. contributed to biochemical analysis, data analysis and preparation of manuscript. The authors have no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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