There be giants!

The importance of taxonomic clarity of the large ocean sunfishes (genus *Mola*, Family Molidae) for assessing sunfish vulnerability to anthropogenic pressures.

Submitted by

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Marianne Nyegaard

Abstract

The study of ocean sunfishes has for decades been practically synonymous with the study of *Mola mola*, recently listed as ‘vulnerable’ on a global scale by the International Union for Conservation of Nature. The concerns are high levels of fisheries bycatch worldwide, however sunfish bycatch is rarely identified to species level anywhere, perhaps as a long legacy of taxonomic confusion in the sunfish taxonomy has rendered identification to species level challenging. This includes the Australian and New Zealand longline fisheries, where sunfishes are listed at “High Risk” due to data deficiency. In the popular sunfish SCUBA dive tourism off Bali, Indonesia, another type of anthropogenic pressure is manifested through diver crowding, preventing sunfish from interacting with cleaner-fish on the local reefs. The consequences are difficult to gauge due to a paucity of information on this highly seasonal phenomenon, but have motivated discussions of sunfish protection by Indonesian authorities.

In this study, the species identities and zoogeographies of the little studied sunfishes in Australia and New Zealand were explored through biopsy sampling in the longline fisheries, and by reviewing museum collections across both countries. Specimen IDs were established phylogenetically and/or morphologically. Combined, the results revealed a new species of ocean sunfish, *Mola tecta*, which was diagnosed and described. Furthermore, the results showed that three large species of sunfish dominate the tropical, subtropical/warm-temperate and cold-temperate waters of Australia and New Zealand; *Masturus lanceolatus*, *Mola alexandrini* and *Mola tecta*, respectively. *Mola mola* appears to be rare. These results imply that the long-term fisheries observer sunfish bycatch data from both countries consists of a mix of species. Bycatch rate analyses within four fishing grounds sub-areas, each presumably dominated by one species of sunfish, did not reveal downwards trends over the 10 - 12 year periods, for which data were available.

In the Bali tourism industry, the sunfish species identity was confirmed molecularly and morphologically as *Mola alexandrini*. The strong seasonality in diver sightings was documented through sunfish encounter rates based on operator logs. The results revealed that the 2015 sunfish season (August – October/November) lagged by ~1 month the seasonal change in intensity of two major oceanographic features of the area, established from *in situ* and satellite sea surface temperature data. Data from eight satellite tagged sunfish revealed a high affinity by four fish to the dynamic Lombok Straight during the sunfish season, however, at least one other fish left the area. Overall, temperature emerged as an unlikely main driver of the sunfish seasonality, which is instead presumably driven by an increase in sunfish prey availability, associated with the seasonal cold-water upwelling.

Overall, these findings have provided much needed clarity to the taxonomy and phylogenetic relationships of the genus *Mola*, and demonstrated the need for species-level investigations of fisheries bycatch globally to inform fisheries risk assessments. Furthermore, the results provided important information on the sunfish seasonality off Bali to inform tourism management decisions.

Statement on the contribution of others

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Contribution to data chapters

Chapter 2 – *Marianne Nyegaard* collected samples, directed volunteers to collect samples, assessed museum specimens, directed museum staff/volunteers to collect information, liaised with museums in Australia, New Zealand, Chile and the Netherlands, undertook phylogenetic and other data analyses and wrote the manuscript; *Joanna Gillum* did the laboratory analysis; *Marianne Nyegaard* undertook the historic literature review in collaboration with *Etsuro Sawai*; *Andrew Stewart* and colleagues processed and prepared the holotype; *Carl Struthers* photographed the holotype and *Michelle Freeborn* made the illustrations. *Andrew Stewart*, *Etsuro Sawai*, *Joanna Gillum*, *Neil Gemmell*, *Neil Loneragan*, *Yusuke Yamanoue* and three anonymous reviewers from the Zoological Journal of the Linnean Society critically reviewed the manuscript.

Chapter 3 – *Steve Hall* (Australian Fisheries Management Authority) and *Stephen Brouwer* and *James Andrew* (New Zealand Ministry for Primary Industries) and fisheries observers collected samples in the longline fisheries in Australia and New Zealand, *Marianne Nyegaard* did the laboratory analysis, phylogenetic and fisheries data analysis and wrote the manuscript. *Etsuro Sawai*, *Mette Nyegaard*, *Neil Loneragan* and two anonymous revivers from Estuarine, Coastal and Shelf Science critically reviewed the manuscript.

Chapter 4 – *Marianne Nyegaard* collected samples, undertook laboratory and phylogenetic analyses, deployed satellite tags and temperature loggers, collected sightings data with the help of volunteers, analyzed the data and wrote the manuscript. *Neil Loneragan*, *Jennifer Verduin*, *Marthen Welly* and *Rili Djohani* critically reviewed the manuscript.

Appendix A3.1 – museum specimens were assessed in person by *Marianne Nyegaard* (Melbourne, Otago, Perth), *Marianne Nyegaard* and *Etsuro Sawai* (Wellington), *Etsuro Sawai* (Auckland), curators or volunteers under instruction from *Marianne Nyegaard* (Whanganui, Hobart, Darwin, Sydney, Adelaide). Strandings were attended by *Marianne Nyegaard*, or volunteers or museum personnel under the instruction of *Marianne Nyegaard*, who put the manuscript together. *Etsuro Sawai* critically reviewed the manuscript; a curator from each museum reviewed and verified the details of their respective specimens.

Thesis publications

The following publications are associated with this thesis:

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Chapter 3 was published as:

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Other publications arising from closely related research:

Sawai E, Yamanoue Y, Nyegaard M, Sakai Y. 2018. Redescription of the bump-head sunfish *Mola alexandrini* (Ranzani 1839), senior synonym of *Mola ramsayi* (Giglioli 1883), with designation of a neotype for *Mola mola* (Linnaeus 1758) (Tetraodontiformes: Molidae). *Ichthyological Research* 65: 142–160.

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Chapter 1 General Introduction

1.1 The Molidae

The ocean sunfishes comprises the Family Molidae within the highly derived and diverse Order Tetraodontiformes. Even among the many curious body forms of the Tetraodontiforms, the sunfishes are readily recognisable by their peculiar appearance of a giant fish head with wings. Their unusual morphology owes in part to a complete lack of a true tail, with elements from the dorsal and anal fins instead forming a rudder-like back-end, termed a “clavus” (Fraser-Brunner, 1951) (Fig. 1.1B). The dorsal and anal fins are near-symmetrical, and situated towards the posterior end of the fish (Fig. 1.1B), providing the means of propulsion (Watanabe and Sato 2008). None of the species have swim bladders. The sunfishes typically attract widespread attention when they occasionally strand, partly due to their large size but also due to their unusual appearance, although they have traditionally been viewed as rare and perhaps somewhat unimportant, sluggish oddities of nature (e.g. Cartamil and Lowe 2004; Watanabe and Sato 2008).

The family consists of three genera. Species in the *Mola* and *Masturus* genera are similar in body form and attain very large adult sizes, growing from minute larvae of a few millimetres, to impressive adults, some over 3 m in total length and weighing over two tons in total body weight (Figure 1.1A – C) (Gudger 1937; Martin and Drewry 1978; Sawai et al. 2018). Species of *Mola* and *Masturus* can be readily distinguished from each other by a characteristic extension on the clavus of the latter (Fig. 1.1.D). The third genus, *Ranzania*, contains one species, which attains a modest maximum length of ~70 – 90 cm (Hutchins 2001; Smith et al. 2010) (Fig. 1.1.E) and furthermore, differs by not possessing the thick layer of collagenous connective tissue in the subcutis, characteristic of the large Molidae species (Tyler 1980).

As a group, the ocean sunfishes occur in all tropical and temperature oceans of the world (Liu et al. 2015), but as they are generally hard to find, handle, store and study, much of the biology is still poorly understood. Older biological research efforts (i.e. ~pre-1950) have typically been based on examinations and observations of small numbers of specimens. Many of these studies approached sunfish research mainly through specimen descriptions of morphology and size, gonads and gut contents and so forth (e.g. Steenstrup and Lütken 1898; Anderson 1900; Schmidt 1921). A large body of work, both historical and contemporary, addresses the taxonomy of the sunfishes (e.g. Block 1785; Ranzani 1839; Fraser-Brunner 1951; Santini and Tyler 2002).

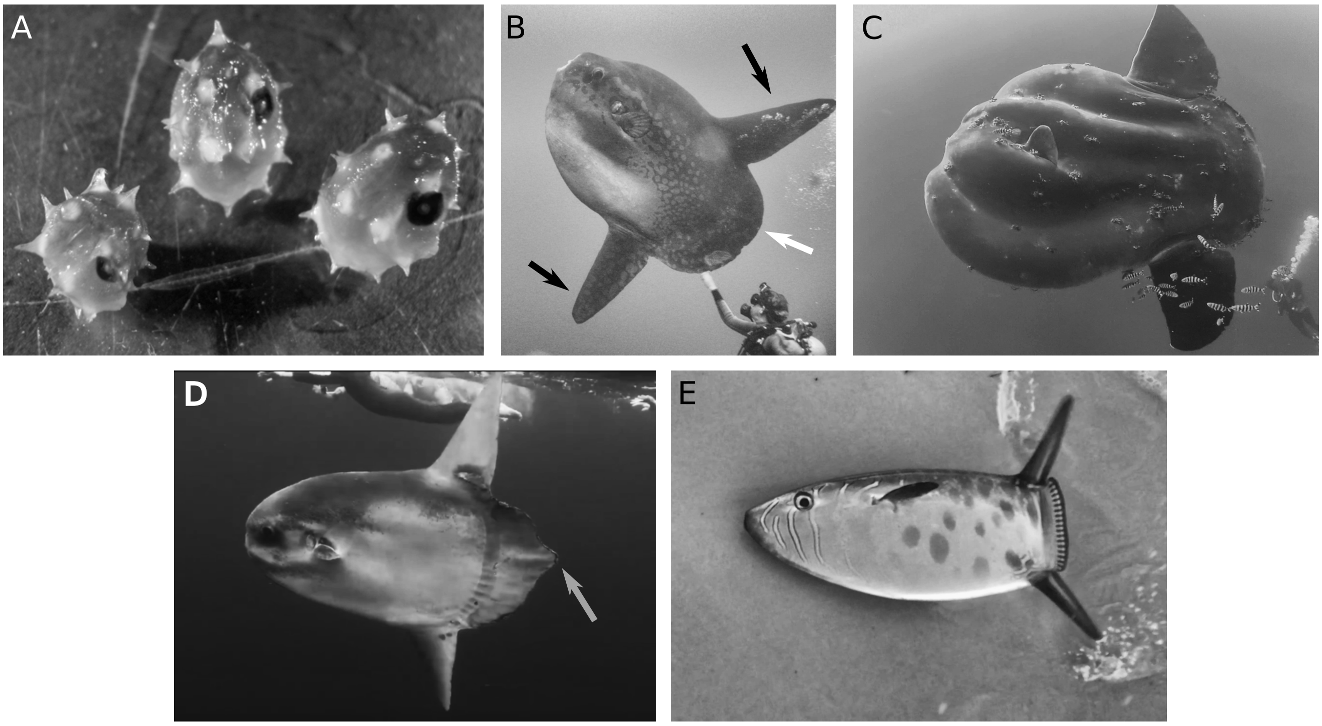


Figure 1.1 **A)** *Mola* sp. larvae (5 – 6 mm total length) in the Australian National Fish Collection, Hobart, Australia; **B)** *Mola alexandrini* off Bali, Indonesia (~1.6 m total length), and **C)** *M. alexandrini* off Gozo in the Mediterranean (unknown total length, possibly > 3 m). Clavus (black arrows) and nearly symmetrical dorsal and anal fins (white triangles). **D)** *Masturus lanceolatus* off Nusa Penida, Indonesia, and **E)** *Ranzania laevis* off Western Australia. Photos by Jonathan Anderson (B), Eric van der Goot (C), Adi Huang (D) and Darren Howlett (E), reproduced with permission.

In recent years, with the development of more sophisticated scientific tools and technologies, sunfish research has taken new and exciting directions. The majority of research has targeted *M. mola* (and species now synonymsed with *M. mola* (Eschmeyer et al. 2018) traditionally considered to be the most common of the sunfishes (Pope et al. 2010; Liu et al. 2015). Traditional diet studies together with the use of genetic and stable isotope analyses, have revealed an ontogenetic shift from a coastal diet by small sunfish (< ~80 – 100 cm), to a gelatinous diet in larger sunfish (Syväranta et al. 2012; Nakamura and Sato 2014; Nakamura et al. 2015; Sousa et al. 2016a). Satellite tagging studies have revealed that sunfish are active hunters, diving deep to several hundred meters to hunt for food in low temperatures (Hays et al. 2009; Dewar et al. 2010; Potter and Howell 2010), seemingly seeking dynamic upwelling systems and marine fronts (Sims and Southall 2002; Thys et al. 2015; Sousa et al. 2016b). They return to the surface to regulate body temperature (Nakamura et al. 2015), as well as to seek parasite cleaning interactions with seabirds (Abe and Sekiguchi 2012; Abe et al. 2012). Research on seasonality (Frafjord et al. 2017; Palsson and Astthorsson 2017) and movements

(Sims et al. 2009a,b; Potter et al. 2011; Hahlbeck et al. 2017) have shown that the distribution and seasonal movements of sunfish may be linked to sea surface temperature (Thys et al. 2015; Sousa et al. 2016b; Phillips et al. 2017). Although many more topics of sunfish biology have been explored and elucidated, a detailed review is beyond the scope here, as emerging evidence of taxonomic uncertainties (see Section 5.3) may require some published information on *Mola mola* to be reviewed in light of potential confusion between species. However, the recent surge in research interest in the sunfishes has increasingly revealed that they are, as a group, not at all rare, but potentially abundant worldwide, and potentially important predators of gelatinous zooplankton globally (Breen et al. 2017; Grémillet et al. 2017).

1.2 Financial value of sunfish, and human pressures

1.2.1 Fisheries

The sunfishes have low commercial harvest value (Liu et al. 2015), with only a small number of targeted fisheries globally, most notably in Taiwan (Liu et al. 2009) and Japan (Sagara and Ozawa 2002; Watanabe and Sato 2008). Their role and value in artisanal fisheries is unclear, but sunfish appear to be caught sporadically in Indonesia, where they are generally released, consumed by the local community, used as bait, or infrequently end up at fish markets (M Nyegaard pers obs).

Sunfish are taken as bycatch in various fisheries across the world. The International Union for Conservation of Nature (IUCN) listed *M. mola* as 'Vulnerable to extinction' globally (Liu et al. 2015), primarily motivated by reports of high levels of sunfish bycatch in certain fisheries, combined with indications of rapid declines in the total bycatch in others. The catch reports of concern included annual estimates of 26,000 individual sunfish caught in the Californian drift gill net fishery (cited as Rand Rasmussen, NMFS Southwest Fisheries Science Center, pers comm in Dewar et al. 2010), 36,450 per annum in the Moroccan driftnet fishery in the Mediterranean (estimate by the IUCN was based on Tudela et al. 2005), and 340,000 per annum in the South African longline fishery (cited as Sims et al. 2009b and Petersen 2005). The IUCN assessment concluded "… *it is likely that other fisheries using these same methods are taking large, but unreported, bycatch of M. mola throughout the majority of its range.*" The assessment highlighted the limited sunfish bycatch data and analysis available, and listed 'harvest level trends' as 'research needed (Liu et al. 2015). *Masturus lanceolatus* was also assessed, but was listed as 'of least concern', due to no known major threats to this species (Leis et al. 2015).

Sunfish are also caught as bycatch in the Australian and New Zealand longline fisheries, where they are routinely released alive (AFMA 2011; MPI 2016b) (Figure 1.2). In the Australian fishery, they are ranked as 'High Risk' as a precaution by the management authority, as a robust risk assessment is hindered by a paucity of information on life history and post-release mortality (Ward and Epe 2008; AFMA 2013, 2014). The sunfish bycatch is managed by the federal Australian fisheries authority (the Australian Fisheries Management Authority, AFMA) through an upper trigger level in total allowable annual interactions. High quality fisheries observer bycatch data has been collected in both fisheries for more than a decade, and provides an excellent opportunity to compare sunfish bycatch rates with those of the South African longline fishery, as well as to investigate trends over time. To date no such detailed analysis has been done.

Figure 1.2 Sunfish caught as bycatch in the Australian and New Zealand longline fisheries, prior to live release. Photos by anonymous fisheries observers.

1.2.2 Nature-based tourism

As with other charismatic marine megafauna (O'Malley et al. 2013; Ward-Paige et al. 2013), sunfish have economic value in nature-based tourism industries. They are, however, seldom the target animals due to their generally sporadic and unpredictable occurrence, but make for exciting, incidental sightings during activities such as cetacean tours (e.g. S Buetow, Wild Ocean Tasmania, pers comm 2018; de Boer et al. 2018), and recreational scuba diving (e.g. RG Sanchez, Buceando Chile, pers comm 2018). In a small number of locations, including the Galapagos Islands (Thys et al. 2017) and the Alboran Coast in the Mediterranean (D Patricio, Hombre y Territorio, pers comm 2018), their occurrence is sufficiently predictable that targeted or semi-targeted sunfish tourism is possible.

Another such place is the Nusa Penida island group near Bali, Indonesia, which is home to a seasonal phenomenon of relatively large sunfish (~1 – 2 m total length; Figure 1.3) seeking out cleaner fish interactions on the shallow reef slopes of Bali and Nusa Penida, at depths accessible to recreational scuba divers (i.e. < 40 m) (Figure 1.3) (Konow et al. 2006; Ruchimat et al. 2013; Thys et al. 2016). The scuba diving tourism on both Bali and the Nusa Penida islands has increased rapidly over the past 2 – 3 decades and diver crowding of the sunfish, which disturbs the sunfish – cleaner-fish interactions, has become a tangible problem in recent years (Figure 1.3) (S Faust, All4Diving; A Taylor Blue Corner Dive; S Bebe, World Diving, pers comm 2018). The increase in tourism boat traffic has also increased the risk of sunfish boat and propeller strikes. There are widespread concerns among tourist operators and non-profit organisations (M Welly, Coral Triangle Center, pers comm 2013 - 16) that sustained harassment may have negative long-term consequences for the sunfish. Currently, the Indonesian government body, the Ministry of Marine Affairs and Fisheries, are reviewing the potential need for national protection of sunfish, partially due to concerns of potential tourism related impacts in the Bali area (M Welly pers comm 2018). There is, however, limited information available to conduct a meaningful risk assessment, and identify what may be effective management strategies. Specifically, there is limited clarity on what drives the strong seasonality of sunfish on the reef systems, exactly when the ‘sunfish season’ occurs and how much of it overlaps with the peak tourism dive pressure.

Bridging some of these gaps was the original major focus of my PhD research, in particular to investigate the likely drivers of the strong seasonality of the Bali sunfish. Furthermore, I intended to investigate bycatch trends in the Australian and New Zealand longline fishery and compare these with the catch rates in the South African longline fishery. As I progressed in my research, however, it became clear that the taxonomic relationships of the sunfishes required urgent attention before it would be possible to focus on the ecological questions of interest.

Figure 1.3 Left: Undisturbed Bali sunfish (*Mola alexandrini*) interaction with cleaner-fish [Longfinned banner-fish (*Chaetodon kleinii*) and adult emperor angle fish (*Pomacanthus imperator*)]; Right: diver crowding of Bali sunfish at Nusa Penida, Indonesia (photograph by Kurt Cotoaga, reproduced with permission).

1.3 Opening Pandora's box of sunfish taxonomy

In 2013, at the inception of this study, Molidae taxonomy had been stable for over 60 years, and consisted of four species in three genera: the small Slender sunfish *Ranzania laevis* (Nardo 1840) the Sharptail sunfish *Masturus lanceolatus* (Liénard 1840), the iconic Ocean sunfish *Mola mola* (Linnaeus 1758) and its lesser known sister species, the Southern sunfish *Mola ramsayi* (Giglioli 1883) (Fraser-Brunner 1951; Parenti 2003a; Eschmeyer et al. 2018).

However, this elegant simplicity hid a messy legacy of taxonomic confusion. Extensive historical interest in the sunfishes had resulted in an immense number of publications in several languages dating back to 16th century European ichthyology, with references to sunfish as far back as the Romans (Gudger and MacDonald 1935; Parenti 2003a; Sawai et al. 2018). After the introduction of Linnaean taxonomy in the 18th century, a plethora of sunfish species were named, and a large, complex web of synonyms and references soon complicated sunfish nomenclature (Fraser-Brunner 1951; Tyler 1980; Parenti 2003b). By the turn of the 19th century, there were over 50 species in 19 genera in the Molidae; years of careful study by two Danish scientists, Steenstrup and Lütken (1898) vividly illustrates the history of the confusing Molidae taxonomy, unfortunately the long and detailed narrative is in antiquated Danish and not widely accessible. It was Fraser-Brunner, who in 1951 reined in the Molidae taxonomy, and suggested that the genus *Mola* consisted of just two species; *M. mola*, widespread across the world's oceans, and *M. ramsayi*, replacing *M. mola* in the South Pacific, where "…the two meet […] in the Australian area". With relatively limited information available at the time on sunfish from the high latitudes of the southern hemisphere, the complicated sunfish taxonomy history was boxed up and stowed away for the next six decades.

In 2013, however, the lid on Pandora's sunfish taxonomy box started to open. Two separate research teams had found four genetic *Mola* clades worldwide, and were considering how these related to *M. mola* and *M. ramsayi*; the only two *Mola* species recognised at the time. One study was based purely on phylogenetics and classified the four genetic clades as Atlantic and Pacific *M. mola* and *M. ramsayi*, respectively (Bass et al. 2005). The other study took both phylogenetics and morphology into consideration, and grouped their findings of four genetic clades into three species (or groups), *Mola* sp. A, *Mola*. sp. B and *Mola*. sp. C (Yoshita et al. 2009). In doing this, they grouped two sister clades into one, *Mola* sp. B, on the basis of similar morphology, and tentatively linked this group to *M. mola*. They also found that *Mola* sp. A superficially resembled *M. ramsayi*, as described by Fraser-Brunner (1951), albeit with one important discrepancy. Specifically, *M. ramsayi* was described as lacking a 'band of reduced denticles between dorsal and anal fin' (*op. cit.*). In turn, the *Mola* sp. A sampled by Yoshita et al. (2009), clearly had a band of reduced denticles. The third clade identified in the study, *Mola* sp. C, was based on sequences from one specimen from New South Wales in Australia and two from South Africa; the latter two were first published by Bass et al. (2005) as the Atlantic clade of *M. ramsayi* (Sawai et al. 2017). However, no morphological information was available from this putative species clade.

This was intriguing, and raised several questions. *Mola ramsayi* was described in 1883 from New South Wales in Australia (Giglioli 1883), where both *Mola* sp. A and *Mola* sp. C had been found by Yoshita et al. (2009). Considering the ambiguity in morphology between *Mola* sp. A and *M. ramsayi*, could *Mola* sp. C be *M. ramsayi*, and *Mola* sp. A another species altogether? And was it possible that – whatever their nomenclatorial statuses – *Mola* sp. A and *Mola* sp. C were so morphologically similar that they had been widely mistaken for each other? Furthermore, were these two genetic clades sympatric with *M. mola*? And if three *Mola* species occurred in Australia, what would that mean for analysis of fisheries observer longline bycatch data, which was collected predominantly as '*M. mola*' or 'unspecified Molidae'?

The species identity of the Bali sunfish did not seem straightforward to resolve either. Although known locally as "mola-mola", and commonly referred to as *M. mola* in the literature (Konow et al. 2006; Ruchimat et al. 2013; Berdej and Armitage 2016) (but see Thys et al. 2016), it was apparent that they did not correspond morphologically to *M. mola* as described by Fraser-Brunner (1951). Instead, they superficially resembled *Mola* sp. A of Yoshita et al. (2009), although a high degree of variation between individuals was evident from the many tourist photos available online through Google searches and on social media platforms.

Importantly for data collection and analysis purposes for this PhD, could the Bali sunfish phenomenon be assumed to consist of just one species? It seemed a risky assumption given the uncertain state of the taxonomy, the potential that some species could have superficially indistinguishable morphologies, and in particular, as a multi-species situation would have major implications for research on sunfish seasonality.

Before my PhD research had even started, I realised that a detour in direction was needed.

1.3.1 Thesis objectives

The overall objective of my thesis became to establish clarity on what species of large ocean sunfishes occur in the Oceania region, specifically in Indonesia, Australia and New Zealand, to better understand how anthropogenic pressures may be assessed on individual species.

This was achieved by focusing on the following discrete aims:

The first major aim was to find and describe *Mola* sp. C, and resolve its nomenclatorial status in the light of the long legacy of taxonomic confusion in the Molidae (Chapter 2).

The second major aim was to establish which species of sunfish occur in Australia and New Zealand, and what species are caught in the longline bycatch, to inform the interpretation of fisheries bycatch trend analysis and assessment of potential risks to sunfish populations in this region (Chapter 3).

The third major aim was to establish which species the Bali sunfish belong to, and if more than one species frequents the reefs, to inform the interpretation of sunfish seasonality based on observational and telemetry data (Chapter 4).

Clarifying the taxonomy of the genus *Mola* (Chapter 2) is significant for understanding the biology and ecology of the ocean sunfishes, as well as for assessing risks to their populations from anthropogenic pressures. Given the legacy of taxonomic confusion in the Molidae, and in particular in the genus *Mola*, new research is urgently required to provide management with species-level information on the large ocean sunfishes in their areas. This will allow for better assessment and management of risks to individual sunfish species from anthropogenic activities, such as the Australian and New Zealand longline fisheries (Chapter 3) and the nature-based tourism off Bali, Indonesia (Chapter 4).

1.3.1.1 Collaboration

Concurrent with my PhD research, a Japanese research group endeavoured to describe further *Mola* sp. A and *Mola* sp. B, and resolve their nomenclatorial statuses. Through collaboration, I was able to compare sunfish material from the Southwest Pacific with material from the Northwest Pacific, and my research greatly benefitted from these insights into the morphology of sunfish from outside my study areas. Furthermore, I was able to compare and discuss my findings in the old taxonomic sunfish literature with those of the Japanese researchers. This proved extremely useful in resolving the complicated matter of tying the genetic *Mola* clades to nomenclature. The Japanese research, which also includes a species level comparison of *Mola* sp. A, *Mola* sp. B and *Mola* sp. C, is described in Sawai et al. (2018).

Chapter 2 Hiding in broad daylight: molecular and morphological data reveal a new ocean sunfish species (Tetraodontiformes: Molidae) that has eluded recognition

2.1 Abstract

The taxonomy of the ocean sunfishes (Molidae) has a complicated history. Currently, three genera and four species are recognized, including two in the genus *Mola* (*M. mola* and *M. ramsayi*). In 2009, a genetic study revealed a potential third species, *Mola* species C, in Southeast Australian waters. Concentrating on this region, we obtained samples and morphological data from 27 *Mola* sp. C specimens, genetically confirmed the existence of this species (mtDNA D-loop and cytochrome *c* oxidase 1), and established its morphology across a size spectrum of 50 – 242 cm total length. *Mola* sp. C is diagnosed by clavus meristics [15 – 17 fin rays (13 – 15 principal, 2 minor), 5 – 7 ossicles, paraxial ossicles separate], clavus morphology (prominent smooth band back-fold, rounded clavus edge with an indent), and body scale morphology (raised conical midpoints, non-branching). This species does not develop a protruding snout, or swollen dorso- or ventrolateral ridges. Body proportions remain similar with growth. A review of the historic literature revealed that *Mola* sp. C is a new, hitherto undescribed species, *M. tecta*, which we describe and diagnose, and that it is the first proposed addition to the genus *Mola* in 125 years. Its core distribution is likely in the temperate waters of the Southern Hemisphere.

2.2 Introduction

The taxonomy of the ocean sunfishes (Molidae Bonaparte 1832) has a long and complicated history dating back more than 250 years, when *Mola mola* (Linnaeus 1758) (originally *Tetraodon mola*) was first placed within the Linnaean classification system. Over the following century, numerous putative new species were described, typically based on single specimens, ranging in life history stages from pre-juveniles (e.g. Koelreuter 1766; Pallas 1770; Mitchill 1828) to large adults (e.g. Ranzani 1839; Giglioli 1883; Philippi 1892). The taxonomy quickly became complex due to limited clarity on intraspecific morphological variation, ontogenetic changes and the potential for sexual dimorphism. This confusion was further compounded by the difficulty in preserving and storing sub-adult and adult specimens. Many specimens were reduced to mounted skins with varying degrees of accuracy in the reproduction of morphology (e.g. Philippi 1892; Harting 1865). Furthermore, opinions differed as to the correct systematic position of these unusual looking fishes, so species were re-grouped and moved between existing and new taxonomic groups, generating a large number of synonyms. Together with spelling deviations, the number of names in the literature for the Molidae soared (e.g. Fraser-Brunner 1951) and by the turn of the 19th century, the cumulative number of nominal genera and species had surpassed 19 and 50, respectively (Parenti 2003; Eschmeyer, Fricke & van der Laan 2017). Comparative reviews of the taxonomy were (and still are) difficult due to descriptions and drawings with limited detail and lack of accuracy (e.g. Linnaeus 1758; Guilding in Swainson 1839; Ayres 1859), the logistics associated with handling, preserving and storing the very large adults (e.g. Steenstrup & Lütken 1898; Matsuura, 2015), and the lack of retention or later loss of holotypes (Parenti 2003; Eschmeyer et al. 2017).

During the 20th century, a general consensus emerged that only a small number of species of sunfish existed. A landmark review of the Molidae by Fraser-Brunner (1951) synonymized the family to just five species in three genera: *Ranzania* Nardo 1840, *Masturus* Gill 1884 and *Mola* Koelreuter 1766. He proposed two species in the genus *Mola*: *M. mola* and *M. ramsayi* (Giglioli 1883), still recognized by major taxonomic authorities today (e.g. Bailly 2015; Eschmeyer et al. 2017). Fraser-Brunner (1951) suggested that *M. mola* had a wide distribution in the world's oceans, but was replaced by *M. ramsayi* in the Southern Pacific, with sympatric distributions around Australia.

In 2009, Yoshita et al., using phylogenetic analysis based on D-loop sequences and accompanying morphological data, identified two clades of *Mola* occurring sympatrically off Japan. They termed them *Mola* group A and B (hereafter termed *Mola* sp. A and *Mola* sp. B), and tentatively linked them to *M. ramsayi* and *M. mola*, respectively, based on matching morphology with Fraser-Brunner (1951). However, one morphological discrepancy between *Mola* sp. A and *M. ramsayi* could not be resolved: a smooth clavus band (termed 'band of reduced denticles' by Fraser-Brunner 1951) was present on *Mola* sp. A (Yoshita et al. 2009), whereas *M. ramsayi* was described by Fraser-Brunner (1951), and several subsequent authors, as lacking a smooth band (e.g. Heemstra 1986; Glover 1994; Hutchins 2001; Bray 2008; Thys et al. 2013). While further research is needed to formally resolve this discrepancy, here we follow Yoshita et al. (2009), and Matsuura (2015) in tentatively linking *Mola* sp. A to *M. ramsayi*, on the grounds that the smooth band was probably overlooked on the *M. ramsayi* holotype, an oversight subsequently repeated in the literature. Previous and recent findings show that *Mola* specimens from New South Wales in Australia, the type locality of *M. ramsayi*, indeed cluster with *Mola* sp. A (Yoshita et al. 2009; M Nyegaard unpublished data). We also follow Yoshita et al. (2009) in linking *Mola* sp. B to *M. mola*, but recognizing further research is needed to resolve the nomenclature of what appears to be an Atlantic and Pacific clade (Ahuir-Baraja et al. 2017; Sawai et al. 2017).

In addition to the *Mola* sp. A and *Mola* sp. B clades, Yoshita et al. (2009) also found evidence of a third *Mola* species (*Mola* sp. C), based on the D-loop sequence of one specimen from New South Wales in Australia, and the partial D-loop sequences of two specimens from South Africa. The latter two specimens were originally from a genetic study by Bass et al. (2005), who interpreted them as a *M. ramsayi* Atlantic group (see Sawai et al. 2017). As no morphological information was collected in either study, the taxonomic status of *Mola* sp. C remained unresolved.

Given the need to formally link genetic clades with nomenclature, BLAST searches of ocean sunfish DNA sequences through the Barcode of Life Data Systems (BOLD; *https://www.boldsystems.org*) present some pitfalls. Over 100 Molidae mtDNA cytochrome *c* oxidase 1 (CO1) sequences are, as of March 2017, lodged with BOLD, forming four BINs: *Ranzania laevis* (Pennant 1776) (comprising the majority of the Molidae sequences), *Masturus lanceolatus* (Liénard 1840) and two separate clusters of *M. mola*. Currently, no CO1 sequences labelled *M. ramsayi* (or *Mola* spp. A, B or C) are available; however, blasting a *Mola* sp. A CO1 sequence returns a small number of very high similarity scores, pointing to potential mislabelled sequences. This is not surprising, given *Mola* sp. A has been mistaken for *Mola* sp. B in various parts of the world (Sagara et al. 2005; Yoshita et al. 2009; Thys et al. 2013; Sawai et al. 2017). In light of recent genetic and taxonomic findings, a review of available Molidae CO1 sequences would therefore be useful, including to verify the existence of *Mola* sp. C as a separate species.

During a recent large-scale Molidae biopsy survey around Australia and New Zealand, we found a subset of individuals among our material, nesting genetically within the *Mola* sp. C D-loop clade of Yoshita et al. (2009). The aim of this study is to formally describe *Mola* sp. C, and this was achieved by first verifying the existence of the clade through analysis of the CO1 locus, comparing our specimens to Molidae sequences available online. After establishing the common morphological characteristics of genetically verified *Mola* sp. C specimens, we reviewed museum material across Australia and New Zealand to locate any *Mola* sp. C specimens held in collections. Based on both fresh and preserved specimens, we describe the morphology of *Mola* sp. C across a size spectrum of 50 – 242 cm total length (TL). We compare the morphology to the original descriptions of the synonyms of *M. mola* and *M. ramsayi* to establish whether *Mola* sp. C has been described previously. On the basis of both genetics and morphology, we conclude that *Mola* sp. C is a new *Mola* species, which we describe herein.

2.3 Material and methods

2.3.1 Specimen collection

A large-scale, non-lethal Molidae biopsy-sampling program was undertaken in 2013 – 2015 in the Australian and New Zealand tuna and billfish long-line fisheries, where sunfish are occasionally caught as bycatch. From 2013 onwards, museums across Australia and New Zealand kept us informed of local sunfish strandings; we obtained photographs, measurements and genetic samples opportunistically, with the help of local residents, fisheries department and museum staff, as well as attending strandings ourselves where possible. To acquire specimens for closer examination, we euthanized three small *Mola* sp. C from the New Zealand long-line fishery and prepared one of these as holotype at the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand (NMNZ).

2.3.2 Review of museum specimens

The Molidae collections at the natural history museums in Australia and New Zealand were reviewed to identify specimens with morphological features akin to *Mola* sp. C. The Melbourne (NMV), Perth (WAM), Auckland (AIM), Otago (OMNZ) and Wellington (NMNZ) museum collections were inspected in person, while material from the Adelaide (SAMA), Darwin (NTM), Hobart (TMAG), Sydney (AMS), Queensland (QM), Christchurch (CMC) and Whanganui (WRM) museums were inspected via photographs and descriptions provided by museum staff [institutional codes follow Sabaj (2016), except OMNZ and WRM]. From accessible material, we identified a small number of specimens and casts with similar characteristics to our genetically confirmed *Mola* sp. C specimens, and morphometric and morphological data were obtained from these. Tissue samples appropriate for genetic analysis were generally not available from museum specimens (but see Table 2.1).

2.3.3 Molecular sequencing

Tissue samples were fixed in RNALater or 95% ethanol. We used a lithium chloride and chloroform salting out method (Gemmell and Akiyama 1996) for DNA extraction. The mitochondrial D-loop and CO1 loci were amplified using MolaA and MolaB primers (Yoshita et al. 2009), and universal fish primers F1 (Ward et al., 2005), respectively. We used Bioline Biotaq polymerase enzyme for the polymerase chain reaction (PCR) with each reaction consisting of 0.2 µL Taq polymerase, 2 µL 10× buffer, 0.8 µL MgCl2, 0.4 µL dNTP (2.5 mM each), 0.8 µL primers, 13.8 µL water and 1 µL template (10 ng/µL). The 20 µL reaction mixtures were subjected to an initial denaturation of 94°C for 60 s, followed by 25 cycles of

94°C for 30 s, 58°C for 40 s and 72°C for 60 s. The PCR products were purified using the PALL AcroPrep96 filter plate 30 kDa kit prior to Sanger sequencing in forward and reverse using an ABI Big Dye Terminator v.3.1 cycle sequencing kit. Sequencing products were run on an ABI 3730xl DNA Analyzer through the Genetic Analysis Service at Otago University, New Zealand. All sequences were uploaded to NCBI with accession numbers provided in Table A2.1.1 (Appendix A2.1).

2.3.4 Phylogenetic analysis

D-loop sequences were assembled in McVector v.14.5.3, and pooled with *Mola* spp. D-loop sequences from Yoshita et al. (2009) (North and Southwest Pacific), Ahuir-Baraja et al. (2017) (Mediterranean) and Sawai et al. (2017) (Indian Ocean), available from the National Center for Biotechnology Information (NCBI). CO1 sequences were pooled with publicly available Molidae CO1 sequences from NCBI and BOLD (Table A2.1.2 in Appendix A2.1). D-loop and CO1 sequences from one *Mola* sp. B (261 cm TL) and four *Mola* sp. A (24, 58, 120 and 120 cm TL, all with smooth clavus bands) from New Zealand were included as controls, and to confirm the *Mola* sp. A clade in the CO1 analysis. To avoid a large number of indels in the alignment, which can be problematic in phylogenetic inference (Warnow 2012), we used *Ma. lanceolatus* as the outgroup in the D-loop analysis, rather than the more divergent *R. laevis*, which was the outgroup for the CO1 analyses. For both loci, the pooled sequences were aligned with ClustalW implemented in MacVector v.14.5.3, using the default settings. The alignments, with a small number of scattered indels for the D-loop, were checked and trimmed.

For both loci, phylogenetic relationships were inferred using maximum likelihood (ML), Bayesian and maximum parsimony (MP) methods, as described below. The most appropriate model of sequence evolution was selected based on the corrected Akaike and Bayesian Information Criteria (AICc and BIC), calculated in jModelTest v.2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012), using the likelihood scores of 88 candidate models with 11 substitution schemes. This included models with equal/unequal base frequencies (+F), with/without a proportion of invariable sites (+I) and with/without rate variation among sites (+G) (four rate categories). We used an ML-optimized base tree, and a Subtree-Pruning-Regrafting (SPR) tree topology search. The AICc and BIC gave similar results for the D-loop, with HKY85 (Hasegawa et al. 1985) +I+G the best fitting model by BIC, and second best fit by AICc. For CO1, HKY+G was the best fitting model based on both BIC and AICc.

2.3.4.1 Maximum likelihood

ML analysis was done in phyML (Guindon and Gascuel 2003), implemented through the online T-REX server (Boc et al. 2012) under the HKY model for both loci. The base frequencies were optimized, and the transition/transversion ratio, gamma shape (in four substitution rate categories) and the proportion of invariable sites (in the D-loop analysis) were estimated by the software. The tree topology was optimized with the 'Best' setting [best of SPR and nearest-neighbour interchange (NNI)] from five random BioNJ start trees, and nodal support assessed by bootstrap from 1000 pseudo-replicates.

2.3.4.2 Bayesian analysis

Posterior probabilities (PP) for the Bayesian analyses were estimated in MrBayes (Ronquist et al. 2012) under the HKY model, with rate variation among sites (in four categories) for both loci, and with a proportion of invariable sites for the D-loop analysis. We used default priors and settings. Two concurrent analyses were run, each with four Markov chains (three heated and one cold) of 10,000 000 (D-loop) and 2,000,000 (CO1) generations, with the chains sampled every 1000 generations. For both loci, convergence between the two concurrent analyses was confirmed by checking that the SD of split frequencies had decreased below 0.01, and that the effective sample size was above 200 after a burn-in of 30% (Tracer v.1.6; Rambaut et al. 2014).

2.3.4.3 MP analysis

For both loci, MP analyses were conducted in Mega v.7 (Kumar et al. 2016), including all sites, with an SPR search algorithm (level 1) and five random starting trees. Nodal support was estimated using 1000 bootstrap pseudo-replicates.

2.3.5 External morphology

The following external characters were assessed whenever possible: (1) body proportions: presence or absence of a 'head bump' (Yoshita et al., 2009; Sawai et al., 2017), and 'chin bump' (Sawai et al. 2017); (2) lateral shape of the head profile: presence or absence of a protruding snout ('forward-projecting snout' in Fraser-Brunner 1951); (3) frontal profile: presence or absence of swollen 'dorsolateral and ventrolateral ridges' on the body (Barnard 1935); (4) clavus shape: rounded or lobed/wavy (Fraser-Brunner 1951; Yoshita et al. 2009; Sawai et al. 2015), with the clavus being the 'movable lobe at the hind margin of the body' (Fraser-Brunner 1951); and (5) smooth band: present or absent, defined as the 'band of reduced denticles [termed 'scales' herein] between dorsal and anal fins' (Fraser-Brunner 1951) (Fig. A2.1.1).

2.3.6 Clavus and fin meristics

We examined the clavus on a subset of fresh and preserved specimens (Table 2.1). The number of clavus ossicles (Fig. A2.1.1) was established by touch on fresh specimens, and by touch and from X-rays, when available, on museum specimens. The number of clavus fin rays (Fig. A2.1.1), and state of paraxial ossicles (Fraser-Brunner 1951), was established in fresh specimens after the clavus was cut off along the posterior margin of the smooth band, and dried for several days. The dorsal, anal and clavus fin ray numbers, and the state of the paraxial ossicles, were determined on museum specimens from X-rays.

2.3.7 Skin structure

The body scales of the sunfishes are modified into small spines or denticles (Cleland 1862; Tyler 1980; Gauldie 1992) and differ between genera in the family Molidae (Katayama and Matsuura 2016), as well as between species in the genus *Mola* (Cadenat 1959; Gauldie 1992; Sawai et al. 2015; Sawai 2016b). We examined the scale morphology on the body and clavus on a subset of fresh, preserved and museum cast specimens (Table 2.1) directly, and through a binocular microscope. A small piece of skin was sampled from two fresh specimens (NZ12 and NZ17) posteriorly of the pectoral fin (Figure 2.1), dried and compared with reference skin samples from *Mola* sp. A and *Mola* sp. B (E. Sawai unpublished data).

2.3.8 Morphometric measurements

External morphometric measurements follow Yoshita et al. (2009) and a subset of Sawai (2016a) (Figure 2.1) and were obtained from fresh and preserved specimens, as well as museum casts (made from fresh specimens), whenever possible (Table 2.1). The holotype was measured both fresh and after the preservation process in formalin and isopropyl alcohol. We pooled measurements from fresh and preserved specimens, as potential shrinkage from isopropyl alcohol storage is unlikely to significantly affect the dimensions of preserved specimens (Larochelle et al. 2016). The measurements were calculated as a percentage of TL and plotted against TL to assess potential change in body shape with size.

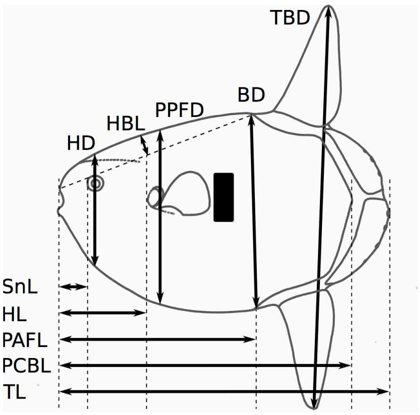


Figure 2.1 Morphometric measurements following Yoshita et al. (2009) and a subset of Sawai (2016a). Body depth (BD), head bump length (HBL), head depth (HD), head length (HL), pre-anal fin length (PAFL), pre-clavus band length (PCBL), pre-pectoral fin depth (PPFD), snout length (SnL), total body depth (TBD), total length (TL). Black box indicates region of skin sampling.

2.3.9 Additional investigations

The digestive tract content of three specimens (Table 2.1) was investigated by emptying the content into a vessel, separating prey items by hand from the heavy parasite load and washing prey items in a 600-μm mesh sieve, as needed, for identification under a binocular microscope.

2.3.10 Review of historical records

To establish whether *Mola* sp. C was among previously described *Mola* species, we reviewed the original descriptions of *M. mola* and *M. ramsayi*, and all nominal Molidae species now considered synonyms of *M. ramsayi* or *M. mola*, or regarded as nomen nudum by Fraser-Brunner (1951), Parenti (2003) and/or Eschmeyer et al. (2017). In total, 37 species descriptions were reviewed, along with relevant, associated literature (Table 2.2).

2.4 Results

2.4.1 Phylogenetic relationship

Details of new *Mola* D-loop sequences (NCBI accession numbers MF158131 – MF158149) are provided in Table A.2.1 (Appendix A2.1). The trimmed alignment of Molidae D-loop sequences (new and from the literature) comprised 816 characters, with 187 parsimony informative sites. The phylogenetic trees inferred by ML, MP and Bayesian methods displayed similar topographies, with no differences in the relationships of the major lineages. As expected, they resembled those of Yoshita et al. (2009), Ahuir-Baraja et al. (2017) and Sawai et al. (2017)

with three major clades: *Mola* spp. A, B and C. Our five control sequences from New Zealand nested within the *Mola* sp. A (*n* = 4) and *Mola* sp. B (*n* = 1) clades, respectively, while the remaining 14 sequences clustered with the *Mola* sp. C sequence of Yoshita et al. (2009) (Figure 2.2). The monophyly of each *Mola* clade was well supported with branch supports of 100% in ML, 1.0 in PP and 100% in MP for each group. The nodal support for the *Mola* sp. A and *Mola* sp. C grouping had moderate support (62% in ML and 71% in MP), with the bootstrap results at times placing *Mola* sp. A and *Mola* sp. B as sister clades.

Details of new CO1 *Mola* sequences (NCBI accession numbers MF158115 – MF158130), and sequences downloaded from BOLD and NCBI, are provided in Appendix A2.1 (Tables A2.1.1 and A2.1.2). The trimmed alignment of the CO1 sequences comprised 627 characters, with 124 parsimony informative sites. Five main clades were produced, each with robust node support of 99–100% in ML, 0.95–1.0 in PP and 91–100% in MP (Figure 2.3). Three clades corresponded to the sequence taxon labels in NCBI/BOLD (i.e. *R. laevis*, *Ma. lanceolatus* and *M. mola*), while a fourth clade comprised our new *Mola* sp. C sequences, ailing with a BOLD sequence labelled 'Tetraodontiformes' (AMS174-08), originating from museum specimen AMS I.41536, registered in OZCAM (2017) as *M. mola*. However, the D-loop from this specimen was included in Yoshita et al. (2009) as sample NNSW-1, that is *Mola* sp. C. The fifth clade comprised our four *Mola* sp. A sequences from New Zealand, and one *Ma. lanceolatus* and two *M. mola* sequences from NCBI/BOLD. These latter three sequences were probably mislabelled for the following reasons: (1) The '*Ma. lanceolatus*' sequence KU945271, from specimen ASIZP0806237 (Chang et al., 2017), was originally identified as *M. mola* in the Fish Database of Taiwan (http://fishdb.sinica.edu.tw), indicating potential sample or specimen identification uncertainties; (2) '*M. mola*' sequence AP006238 is the CO1 locus from the '*M. mola*' mitogenome (Yamanoue et al., 2004), reidentified as *Mola* sp. A (sample OI-1) by Yoshita et al. (2009); and (3) The '*M. mola*' sequence HQ167728 originated from a Mediterranean specimen, identified at the time by its 100% similarity with the CO1 locus of the '*M. mola*' mitogenome AP006238, and a lack of *M. ramsayi* records from the area (E Keskin, Ankara University, pers comm July 2014). However, mitogenome AP006238 was from *Mola* sp. A (see above), and *Mola* sp. A has since been recorded in the Mediterranean (Ahuir-Baraja et al., 2017). We conclude that the mixed cluster in Figure 2.3 is an artefact of sequence mislabelling, rather than an indication of fundamental problems with the *Mola* phylogeny. As such, the CO1 analysis corroborates the D-loop results, with three clear *Mola* species clades, *Mola* sp. B (*M. mola*), *Mola* sp. A (presumably *M. ramsay*) and *Mola* sp. C, showing the latter two as sister clades.

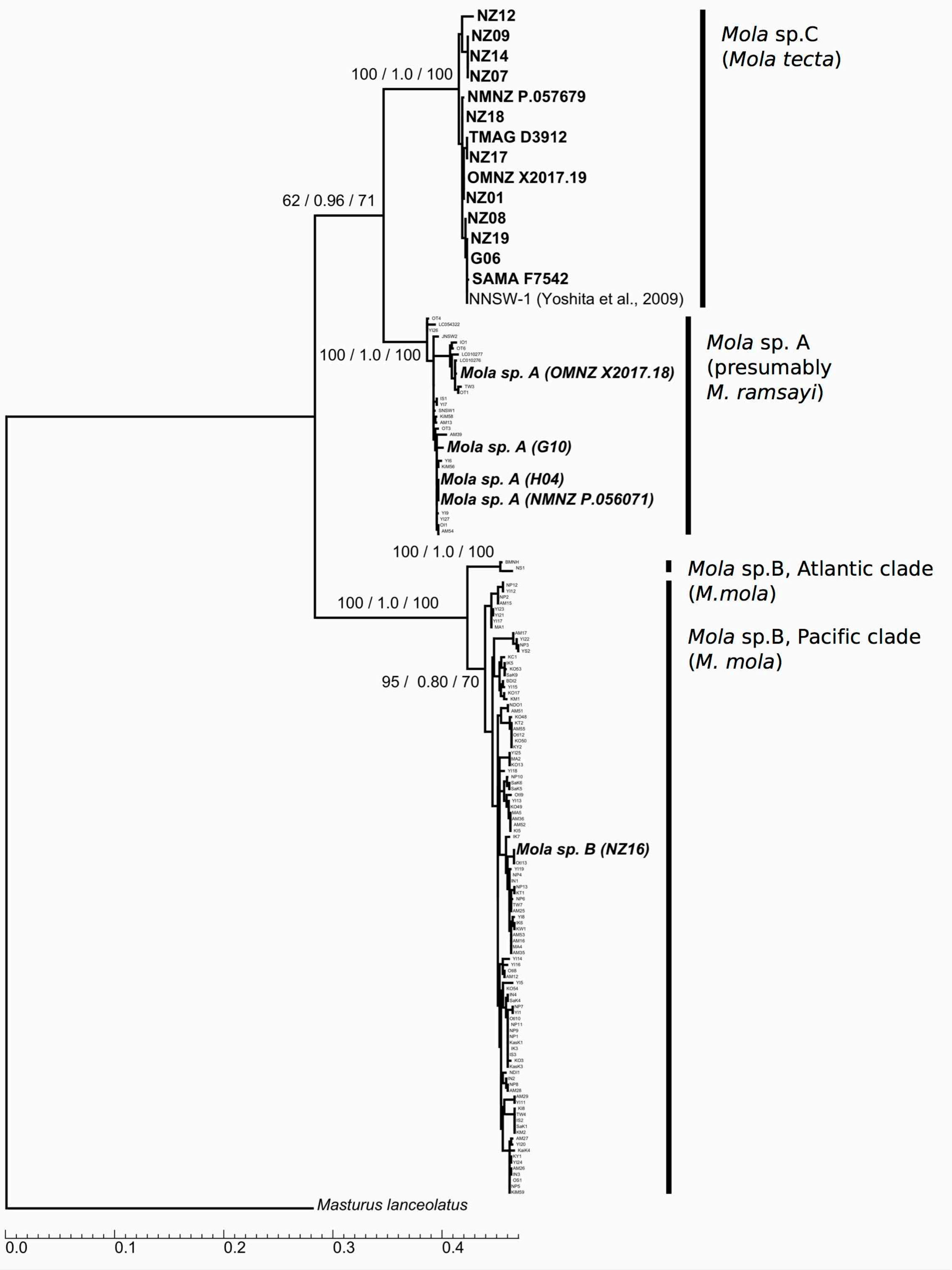


Figure 2.2 Phylogenetic relationships inferred by maximum likelihood (ML), based on *Mola* mitochondrial D-loop sequences from Yoshita et al. (2009), Ahuir-Baraja et al. (2017), Sawai et al. (2017) and this study (in bold). Numbers above or below internal branches of the major clades of *Mola* sp. A, *Mola* sp. B, and *M. tecta* (*Mola* sp. C) refer to ML bootstrap values (left), Bayesian posterior probabilities (middle) and maximum parsimony bootstrap values (right). Scale bar represents nucleotide substitution.

Figure 2.3 Phylogenetic relationships inferred by maximum likelihood (ML), based on Molidae mitochondrial cytochrome *c* oxidase 1 sequences from the Barcode of Life Data Systems, the National Center for Biotechnology Information and this study (in bold). Numbers above or below internal branches of the major species clades refer to ML bootstrap values (left), Bayesian posterior probabilities (middle) and maximum parsimony bootstrap values (right). Scale bar represents nucleotide substitution. *Lodged as Tetraodontiformes; from specimen AMS I.41536, also NNSW-1 in Yoshita et al. (2009), i.e. *Mola* sp. C. **Probably *Mola* sp. A (see text). ^Sample OI-1 in Yoshita et al. (2009), i.e. *Mola* sp. A. ^^Probably *Mola* sp. A (see text); *^Lodged as Tetraodontiformes, from specimen NMV A25071, *Mola* sp. in OZCAM (2017).

2.4.2 Description

Family Molidae Bonaparte 1832

Genus *Mola* Koelreuter 1766. Type specimen *Mola aculeata*, i.e. pre-juvenile form.

Mola Cuvier 1798. Type specimen *Tetraodon mola* Linnaeus 1758, i.e. adult form.

Diagnosis: Genus diagnosis, modified from Parenti (2003): Body ovoid, highly compressed laterally; lacking a true caudal fin; caudal region with a pseudocaudal (clavus), rounded, sometimes slightly crenulated, but without a mid-flap extension. Mouth small; teeth united, fused and beaklike. Fins without spines; dorsal fin posterior, erect, high, short-based, anal fin opposite the same shape. Pelvic fins absent. Skin leathery and thick; scales reduced, rounded base with a single, erect point or rectangular shape; non-imbricated. Gill openings small, oval, in front of pectorals. Lateral lines around eyes (nearly imperceptible). No swim bladder. Darker above, paler or dusky below, often spots and patterns on the sides.

***Mola mola* (Linnaeus 1758)**

Mola mola (Linnaeus 1758). No known holotype (Parenti 2003; Eschmeyer et al. 2017)

Distribution: Currently thought to be widely distributed in all the world's oceans except for the polar seas, however may be relatively uncommon in some areas (e.g. around Australia and New Zealand).

***Mola ramsayi* (Giglioli 1883)**

Mola ramsayi (Giglioli 1883). Holotype: BMNH 1883.11.29.22.

Distribution: Previously thought to be restricted to the South Pacific (Fraser-Brunner 1951), however if *Mola* sp. A is shown to belong to *M. ramsayi*, the distribution is much wider than previously thought, including the Pacific and Indian oceans in both hemispheres, as well as the Mediterranean and probably also the European seas.

***Mola tecta* sp. nov**

(Table 2.1; Figure 2.4–Figure 2.10, Figure 2.12).

urn:lsid:zoobank.org:act:0AE4167C-A2FE-4E77-BD45-B0D465410988.

New English name: Hoodwinker ocean sunfish. New Japanese name: Kakure-manbo.

Orthragoriscus mola (not of Linnaeus) – Lidth de Jeude 1890: 189–190, Pl. 8.

Mola ramsayi (not of Giglioli) – Gauldie 1992: 263–266 [description of skin structure], figs 4 – 6.

– Bass et al. 2005: 405–413 [as 'Atlantic group'], figs 2, 3, table 1; accession numbers AY940816 and AY940826 (partial D-loop); AY940834 and AY940838 (cyt-b).

Mola group C Yoshita et al. 2009: 237, NNSW-1 in table 1 and figs 3, 4; Accession number AB439108 (D-loop).

Mola species C of Yoshita et al. 2009 – Ahuir-Baraja et al. 2017: 1133, table SI, fig. 2 – Sawai et al. 2017: 99 – 102, figs 1, 2, table 1.

Holotype: **NMNZ P.057679** (Figure 2.4) (fresh measurements: 101.1 cm TL; measurements after fixation: 101.2 cm TL), male, North Taranaki Bight, west coast North Island, New Zealand (38°25.5′S 174°9.0′E), trawl, 78 m, 25 December 2015; coll.: observer Scott Yeoman FV Ivan Golubets, OBS 4552/050. Measurements (fresh and after fixation) provided in Table A2.1.3 (Appendix A2.1).

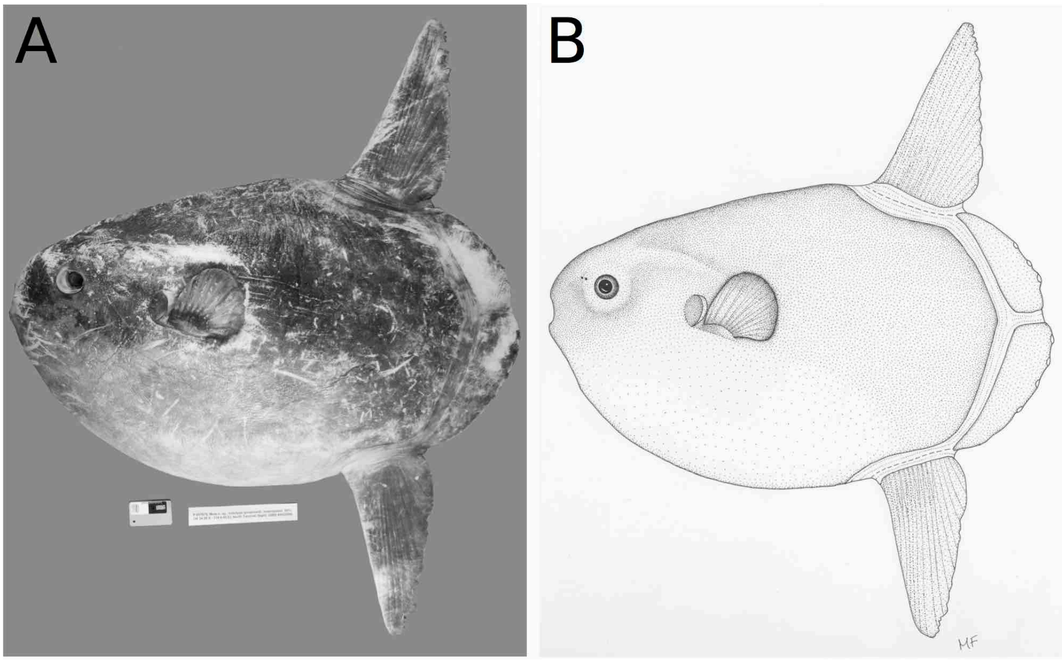


Figure 2.4 *Mola tecta* sp. nov. holotype: NMNZ P.057679, 101.1 cm total length (fresh measurement), male, North Taranaki Bight, west coast North Island, New Zealand (38°25.5′S 174°9.0′E), trawl, 78 m, 25 December 2015, collected by Scot Yeoman, Ministry of Primary Industries, New Zealand. **A)** Photographed and **B)** illustrated by Carl Struthers and Michelle Freeborn, respectively, Museum of New Zealand Te Papa Tongarewa

Paratypes: Eleven specimens (49.9–90.5 cm TL): **AIM MA29864** (51.1 cm TL), female, whole specimen in isopropyl alcohol, Poor Knights Islands, New Zealand (35°29.3'S 173°43.7'E), 8 November 1969; **NMNZ P.001418** (60.2 cm TL), male, whole specimen in isopropyl alcohol, off Hawke's Bay, New Zealand (39°25′S 177°6′E), June 1952; **NMNZ P.002980** (64.6 cm TL), male, whole specimen in isopropyl alcohol, North Rona Bay, Eastbourne, Wellington Harbour, New Zealand (41°16′S 174°55′E), drag net, coll.: Robert Falla, 30 November 1960; **NMNZ P.005890** (49.9 cm TL), female, whole specimen in isopropyl alcohol, off Great Barrier Island, New Zealand (36°5′S 175°35′E), trawl 36–37 m, coll.: C Gaelic, 25 June 1973; **NMNZ P.006126** (64.8 cm TL), female, whole specimen in isopropyl alcohol, Oriental Bay, Wellington Harbour, New Zealand (41°17.40′S 174°47.587′E), beach cast, 29 June 1955; **NMNZ P.033995** (57.2 cm TL), male, whole specimen in isopropyl alcohol, Bay of Plenty, Opotiki Beach, New Zealand (37°45′S 177°20′E), November 1996; **NMNZ P.034187** (79.7 cm TL), male, whole specimen in isopropyl alcohol, Bay of Plenty, surf at Opotiki, New Zealand (37°45′S 177°20′E), hand, coll.: Andy Glazier; **NMNZ P.034217** (69.8 cm TL), male, whole specimen in isopropyl alcohol, Bay of Plenty, Opotiki Beach, New Zealand (37°44′S 177°20′E), coll.: Andy Glazier, December 1996; **NMV A18725** (90.5 cm TL), whole specimen in isopropyl alcohol, Port Phillip Bay, Victoria (37°52′S 144°49′E), coll.: F Hadathy and S Praljak, 28 August 1995; **NMV A26565-001** (78.5 cm TL), whole specimen in isopropyl alcohol, Barwon Heads, Victoria (38°17.4′E 144°27.36′S), coll.: Warren Chapman, 30 April 2009; SAMA F7542 (*c*. 90 cm TL), whole specimen in ethanol, Spencer gulf, South Australia (34°47.4′S 138°28.8′E), coll.: J Verrier, 27 June 1994 [current specimen label indicates 1989, considered an error by collection authority (R Foster, SAMA, pers comm, February 2017)].

Other material: Sixteen specimens (62 – 242 cm TL): **AIM MA30933** (102 cm TL), cast from fresh specimen, New Zealand; **G06** (62 cm TL), live specimen (not retained), northeast of North Island, New Zealand, (36°30′S 177°45′E), long-line, 8 July 2013; **NZ01** (212 cm TL), fresh specimen (not retained), Otago Harbour, New Zealand (45°49′S 170°37′E), beach cast, 18 February 2015; **NZ07** (100 cm TL), fresh specimen (not retained), Banks Peninsula, New Zealand (43°50′S 172°40′E), beach cast, 30 April 2014; **NZ08** (151 cm TL), fresh specimen (not retained), Banks Peninsula, New Zealand (43°50′S 172°40′E), beach cast, 30 April 2014; **NZ09** (193 cm TL), fresh specimen (not retained), Banks Peninsula, New Zealand (43°50′S 172°40′E), beach cast, 30 April 2014; **NZ12** (155 cm TL), female, fresh specimen (clavus, skin sample retained), Birdling’s Flat, Banks Peninsula, New Zealand (43°49′S 172°42′E), beach cast, 10 May 2014; **NZ14** (170 cm TL), fresh specimen (not retained), near Birdling’s Flat, Banks Peninsula, New Zealand (43°50′S 172°40′E), beach cast, 14 December 2015; **NZ17** (81 cm TL), male, fresh specimen (clavus, skin sample retained), west of South Island, New Zealand (41°32′S 170°56′E), purse seine, 12 April 2014; **NZ18** (65 cm TL), female, fresh specimen (clavus retained), east of North Island, New Zealand (38°47′S 178°25′E), long-line, 17 May 2014; **NZ19** (69 cm TL), male, fresh specimen (clavus retained), east of North Island, New Zealand (35°9′S 176°3′E), long-line, 10 August 2014; **OMNZ VT3249** (78 cm TL), cast from fresh specimen, Kaka Point, Clutha District, New Zealand, (46°22′S 169°44′E), beach cast, 7 March 1963; **OMNZ VT3248** (242 cm TL), cast from fresh specimen, Otago Harbour, Dunedin, New Zealand (45°49′S 170°38′E), beach cast, 1961; **OMNZ X2017.19** (169 cm TL), female, fresh specimen (not retained), North Taranaki Bight, New Zealand (38°26′S 174°9′E), beach cast, 18 January 2017; **RMNH. PISC.D.2059** (223 cm TL), female, mounted skin, Ameland, Netherlands, beach cast, 13 December 1889; **TMAG D3912** (150 cm TL), fresh specimen (not retained), Lindisfarne, Tasmania, Australia (42°51′S 147°20′E), beach cast, 12 December 2014.

Diagnosis: Within the investigated size range (50 – 242 cm TL), *M. tecta* sp. nov. is diagnosed from others in the genus *Mola* by the following combination of characters: slender body shape without head bump or chin bump; tapered profile when viewed from the front, with the widest point across the eye area; short dorsolateral ridge to posterior end of pectoral fin; rounded profile without protruding snout. Pronounced smooth band consisting of creased, elastic skin with highly reduced density of body scales; distinctive smooth band back-fold dividing the clavus into a smaller upper and larger lower portion. Rounded clavus, slightly crenulated, with an indentation associated with the smooth band back–fold. Usually 11 – 13 pectoral fin rays, 17 – 19 dorsal fin rays, 16 – 18 anal fin rays, 15 – 17 clavus fin rays (13 – 15 principal rays, 2 minor rays along the edges of the smooth band back-fold); usually 5 – 7 separate ossicles on the clavus edge in specimens over 65 cm TL; no ossicles or ossification associated with the smooth band back-fold, ossicles on paraxial fin rays separate, of similar size to the neighbouring clavus ossicles. Body scales with conical, non-branching erect central point of differing sizes.

Description: Description of the holotype (Figure 2.4) followed by a range of paratypes and sample specimens in brackets (see Table 2.1 for the contribution of data from individual paratypes and sample specimens).

A species of *Mola* with the typical characteristics for the genus, most notably a deep body, highly compressed laterally, truncated, lacking a true caudal fin, near-symmetrical, spineless dorsal and anal fins. Eyes small, round; two small nostrils located in front of eye; mouth small, terminal; teeth fused, beaklike; gill openings small, oval, located in front of the pectoral fins, and covered by a soft gill membrane; gill rakers are concealed under the subcutaneous gelatinous layer; all fins are spineless; caudal fin and pelvic fin absent; pectoral fin small, round, located mid-laterally, fitting into a shallow groove in the side of the body; caudal fin replaced by a clavus, comprised of highly modified elements of the dorsal and anal fins; body covered in thick subcutaneous gelatinous layer except for pectoral, dorsal and anal fins, skin covered in thick mucus, anal opening immediately anterior of anal fin.

No head bump (0), no chin bump (0), no swollen dorsolateral or ventrolateral ridges on body (0); short dorsolateral ridge to posterior end of pectoral fin reflecting the underlying cranio-skeletal structure, front profile tapered (in all investigated specimens) (Figure 2.5, Figure 2.6). Lateral profile rounded, without protruding snout (0) or any indications thereof (0) (Figure 2.5B). Holotype of typical body dimensions (Figure 2.7); *M. tecta* maintains its body proportions with growth, with similar length and depth measurements as percentages of TL across the investigated size spectrum; total body depth (TBD) relative to TL appears to decrease with growth, due to shorter dorsal and anal fins relative to TL in larger specimens (Figs 6, 7A). Eleven (11 – 13) pectoral fin rays, 18 (17 – 18) dorsal and 17 (16 – 18) anal fin rays, 15 (13 – 15) regularly spaced principal clavus fin rays, with 7 (5 – 7) above, and 8 (7 – 8) below the smooth band back-fold. An additional 2 (2) minor fin rays embedded along the edges of the smooth band back-fold; minor fin rays and back-fold positioned between the two paraxial fin rays (Figure 2.8). Seven (0 – 7) ossicles: 3 (0 – 3) above, and 4 (0 – 4) below the smooth band back-fold (Figure 2.4, Figure 2.5C), positioned on the crest of slight crenulations on the clavus trailing edge (Figure 2.8B,C), adult ossicle number established around 65 cm. Paraxial ossicles separate, situated on either side of the smooth band back-fold (in all investigated specimens) (Figure 2.8).

Shape of scales is similar across much of the body and clavus. For comparison with other Molidae, we refer here to the skin on the body posterior of the pectoral fin (Figure 2.1). Scales with a raised central point, which is smooth and conical without branching tips (Figure 2.9). The scales butt against each other; the boundaries become highly visible in the dried state (not shown). Size of the raised, central point on the body scales vary, with larger scattered between smaller (Figure 2.9A, B). Smooth band nearly free of scales (in all investigated specimens); scales only slightly reduced, and of smaller sizes, on the elastic skin of the smooth band back-fold (density of scales on back-fold varies between individuals). Clavus rounded with an indent at smooth band back-fold (Figure 2.4, Figure 2.5C) (back-fold and indent may be less evident in small, preserved specimens due to stiffening of the back-fold during preservation).

Coloration of holotype after defrosting uniform grey, darker dorsally, lighter ventrally (Figure 2.4). The colour of live specimens appears grey or dark brown with dusky white, mottled spots and patterns (Figure 2.6A; Figure A2.1.2 in Appendix A2.1). Darker dorsally, lighter ventrally. It is not known whether live *M. tecta* can rapidly change the contrast of its skin patterns, as *Mola* sp. A and *Mola* sp. B can (M Nyegaard and E Sawai, pers obs), but we consider it likely. The colour of freshly dead specimens is similar to live specimens, or uniform grey to grey-blue (Figure 2.5, Figure 2.6B–E).

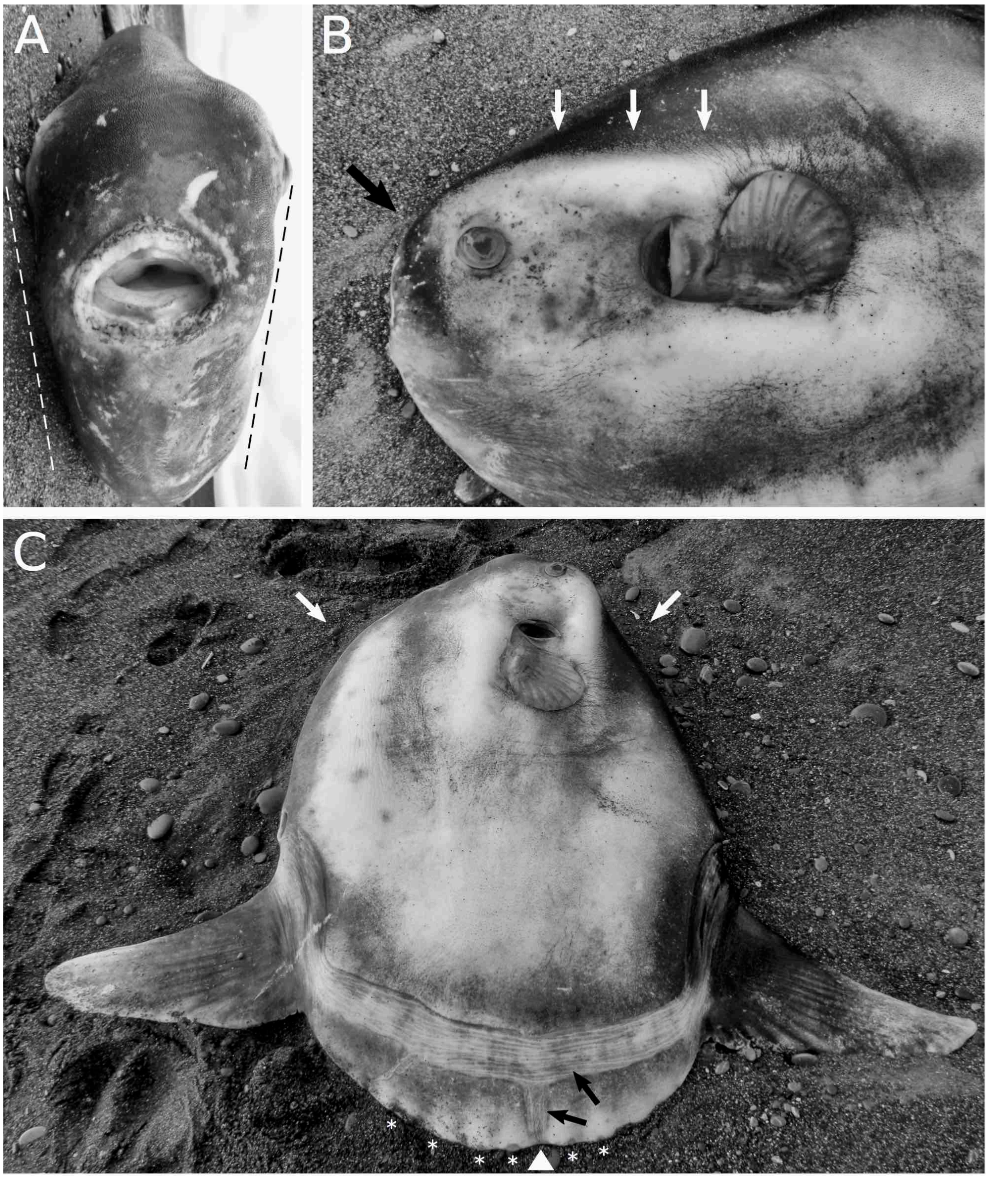


Figure 2.5 *Mola tecta* sp. nov. specimen NZ12, 155 cm total length. **A)** Tapered profile (hashed lines) with widest point across the eye area; **B)** rounded profile without protruding snout (black arrow); short dorsolateral ridge (white arrows) to pectoral fin; **C)** prominent smooth clavus band with back-fold (black arrows), and associated indent (white triangle) on an otherwise rounded clavus edge. White stars indicate ossicles.

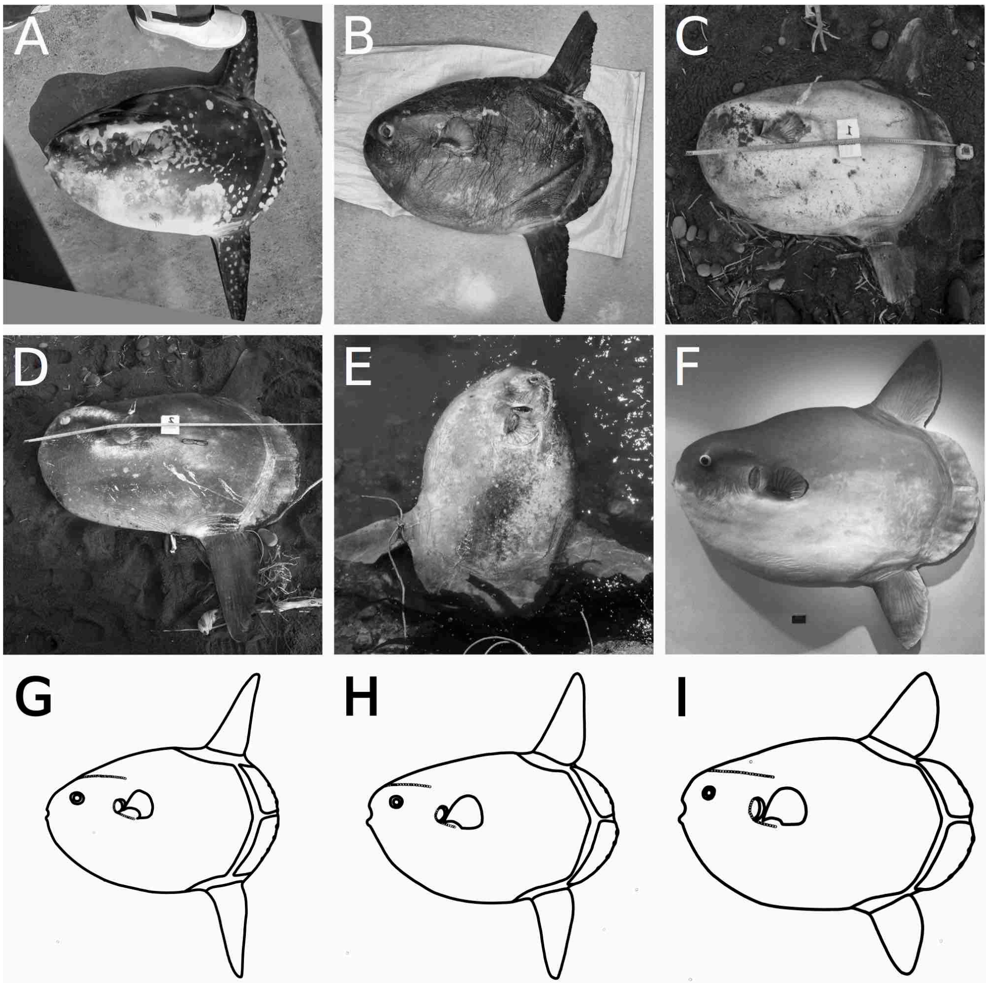


Figure 2.6 Slender body proportions without head bump, chin bump or protruding snout of *Mola tecta* sp. nov. at different total lengths: **A, G)** G06: 62 cm; **B)** NZ18: 65 cm; **C)** NZ07: 100 cm; **D)** NZ08: 151 cm; **E)** NZ01: 212 cm; **F, I)** OMNZ VT3248 242 cm (cast); **H)** NMNZ P.057679 (holotype): 101 cm. Photographs by Tom Trnski, Auckland War Memorial Museum (A), Marianne Nyegaard, Murdoch University; (B,F), Ken Logan, local resident of Christchurch, New Zealand; (C,D), Jean McKinnon, University of Otago (E). Illustrations by Michelle Freeborn, Museum of New Zealand Te Papa Tongarewa (G – I).

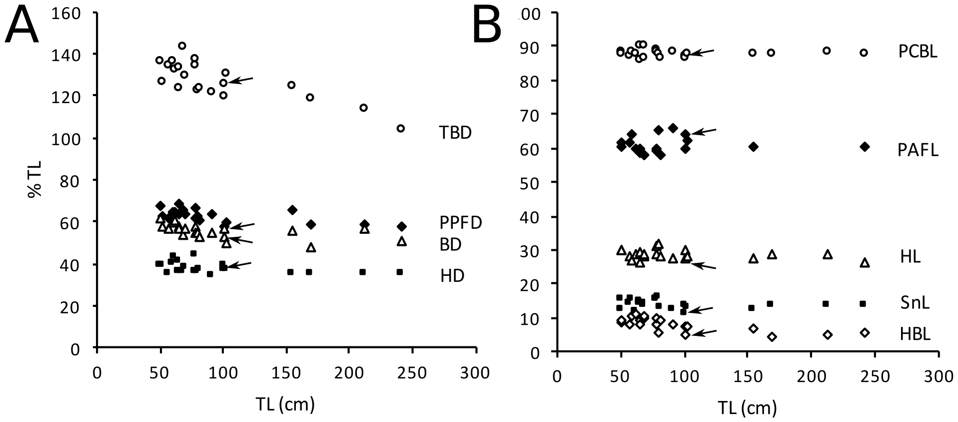


Figure 2.7 Morphometric measurements of *Mola tecta* sp. nov. specimens (Table 2.1), as percentage of total length (TL). **A)** Black diamonds: body depth (BD), black squares: head depth (HD), open triangles: pre-pectoral fin depth (PPFD), open circles: total body depth (TBD); **B)** open diamonds: head bump length (HBL), open triangles: head length (HL), black diamonds: pre-anal fin length (PAFL), open circles: pre-clavus band length (PCBL), black squares: snout length (SnL). Holotype NMNZ P.057679 morphometrics (fresh) indicated with arrows.

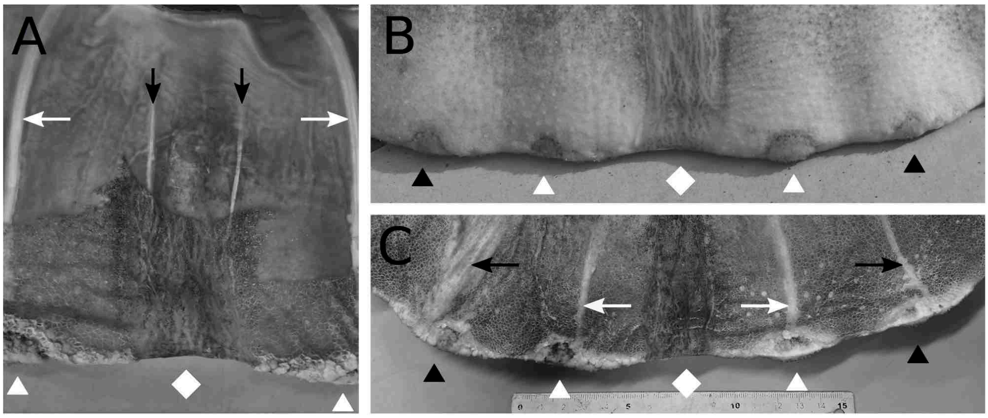


Figure 2.8 Clavus structures of *Mola tecta* sp. nov. specimen NZ12, 155 cm total length. **A)** Dried clavus, left side, skin partially removed: two minor fin rays (black arrows) embedded along the edge of the smooth band back-fold (white diamond) between the paraxial fin rays (white arrows), which bear separate ossicles (white triangles). **B)** Fresh and **C)** dried state, right side: small ossicles (triangles) on the crests of slight crenulations along the rounded clavus edge; no ossicles or ossification associated with the clavus band back-fold (white diamond); paraxial fin rays (white arrows) each bearing a separate ossicle (white triangles), similar to the ossicles (black triangles) on neighbouring principle fin rays (black arrows).

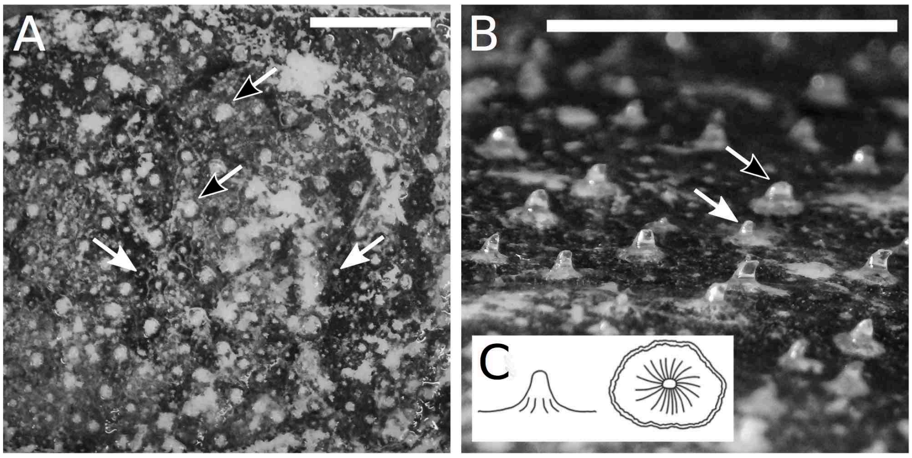


Figure 2.9 Scales of *Mola teca* sp. nov. (holotype NMNZ P.057679, fresh state) on the body behind the pectoral fin (see Figure 2.1) from **A)** a 90 degree angle, and **B)** an oblique angle (1 cm scale bars), with conical, non-branching central points, larger (black arrows) scattered among smaller (white arrows) scales. **C)** Typical shape of *Mola tecta* sp. nov. body scale: side view (left), and 90 degree angle (right), showing the raised central point on the dermal plate.

Etymology: The species name tecta is derived from the Latin tectus (disguised, hidden), as this species evaded discovery for nearly three centuries, despite the keen interest among early sunfish taxonomists and the continued attention these curious fish receive. The Japanese name is derived from the species name tecta: 'hidden' (Kakure), 'sunfish' (manbo), while the English name, 'Hoodwinker', pertains to the figurative meaning 'trickster, deceiving by disguise', *c.* 1600.

Habitat and distribution: *Mola tecta* has been confirmed in the southeast of Australia (New South Wales, South Australia, Tasmania and Victoria) (Yoshita et al. 2009; this study), around New Zealand (this study), and off South Africa (Bass et al. 2005) (Figure 2.10). Photographs (e.g. Figure A2.1.2 in Appendix A2.1) indicates that it also occurs in Chilean waters. This suggests that *M. tecta* is distributed widely in the temperate waters of the Southern Hemisphere, with occasional, but presumably rare, occurrences in the Northern Hemisphere (see below under Broader Molidae literature). Migrations are unknown.

Biology: It attains at least 242 cm TL, probably more. It likely exhibits a similar biology to the other *Mola* species whereby feeding takes place during deep dives (e.g. Nakamura et al. 2015; Thys et al. 2016). Digestive tract contents of three specimens (Table 2.1) consisted of salps (*Thetys vagina* and *Pyrosoma* sp.), and the remains of a nectonic siphonophore, and in one instance, a 3 × 5 mm Styrofoam ball. All dissected specimens were heavily infested with parasites, particularly in the intestines (cestodes) and in the liver (likely larval *Trypanorhynch* cestodes). The shape of the gonads differs between the sexes; the ovary is singular and ballshaped, the testis are paired, elongated and rod-like and do not appear to frill during maturation. Spawning grounds, eggs, larvae and pre-juveniles are not known.

Remarks: The clavus fin ray structure of NZ19 (Table 2.1) appeared abnormal with a secondary, irregular smooth band back-fold present below the main back-fold. Also, in addition to 14 regularly spaced principal fin rays, we found 5 smaller, less rigid and highly irregular fin ray-like structures; 4 of these were set close to each other, bending towards the main back-fold, while the fifth was Y-shaped and associated with the secondary back-fold. Their irregularity and disordered appearance pointed to malformations, which are not uncommon in the other *Mola* species (Sawai et al. 2009). The formation of ossicles (8) also appeared affected.

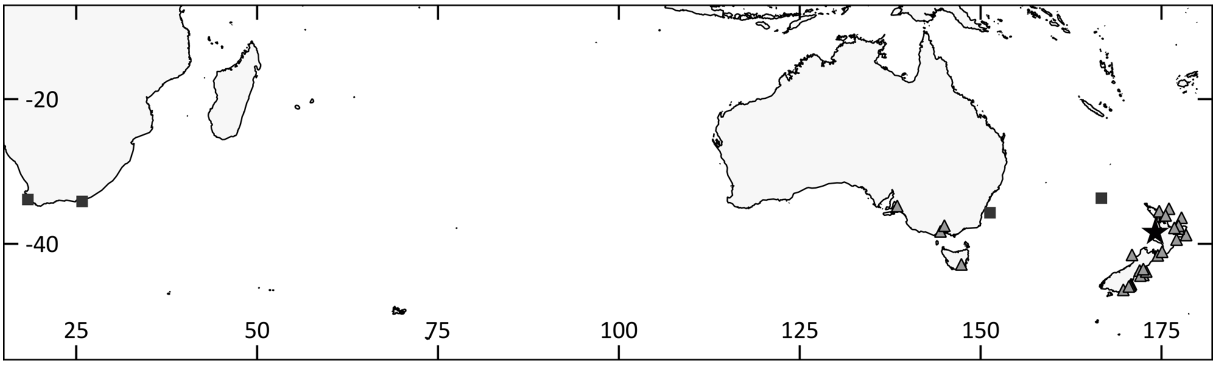


Figure 2.10 Locations of *Mola tecta* sp. nov.: holotype (black star); paratypes and other examined material (excluding RMNH.PISC.D.2059 from the Dutch Coast) (grey triangles); NNSW-1 (Yoshita et al. 2009), AY940816, AY940826 (Bass et al. 2005) and one unnamed specimen from Gauldie (1992) (black squares).

2.4.3 Key to the Mola species

Fraser-Brunner's (1951) review included a key to the genus *Mola*, whereby *M. mola* and *M. ramsayi* were distinguished based on six characters: the number of clavus fin rays and ossicles, the size of clavus ossicles against the space between them, the clavus shape (lobed or rounded), state of the paraxial ossicles (separate or united) and presence/absence of a smooth band. While research in recent years has added important morphological insights into the sunfishes, the outstanding tasks of formally linking the genetic *Mola* sp. A clade with nomenclature, and clarifying the status of the two *Mola* sp. B clades (Atlantic vs Pacific), currently limit us to an interim update of the Fraser-Brunner (1951) *Mola* key. Further research is also needed to determine at what size specific characters are established, for example the number of ossicles, wavy clavus, etc. Thus, the interim key below should be applied with caution, in particular to smaller individuals. Characteristics, which we consider to be generally established at 65 – 70 cm TL, include clavus fin ray numbers, clavus ossicle numbers, state of the paraxial ossicle(s) and the presence of a smooth band back-fold and associated clavus edge indent in *M. tecta*. Also, the scale morphology appears to be fully developed for *M. tecta*, while further research is needed for the other *Mola* species.

Interim key to the species of adult form *Mola* [modified from Fraser-Brunner (1951)]:

> Smooth band (pre-clavus) with pronounced back-fold; clavus supported by 15 – 17 rays [13 – 15 principal, 2 minor], 5 – 7 of the principal rays bearing ossicles; osscicles borne on paraxial rays separate, of similar size to neighbouring; clavus edge rounded, with an indent at the smooth band backfold; without a protruding snout, head bump or chin bump; short dorsolateral ridge to posterior end of pectoral fin; conical body scales of varying sizes, without branching tips, sparsely set …………………………*Mola tecta* sp. nov.
>
> – Smooth band without pronounced back-fold, or with a faint, superficial back-fold; other shape of clavus; 8 or more ossicles ……………………………………………………………....2
>
> Smooth band (pre-clavus) subtle, or moderately pronounced; clavus supported by 14–17 rays, 8–15 of which bear ossicles; clavus edge rounded; protruding snout in some individuals; head bump, chin bump, swollen dorso- and ventrolateral ridges in large individuals; body scales with slightly raised rectangular midpoints in horizontal rows……..……………………………………………………..*Mola* sp. A (presumably *Mola ramsayi*)
>
> – Smooth band pronounced; clavus supported by 10–13 rays, 8–9 of which bear ossicles; ossicles invested with cuticle, which grows to form lobes with fish growth, creating a wavy clavus edge; conical body scales with branching tips, densely set; protruding snout in some individuals; without or with moderate head bump; without or with small chin bump; swollen dorso- and ventrolateral ridges in large individuals ……………………………..
> ……………………………..………………………………………..……….. *Mola* sp. B (presumably *Mola mola*)

Source information for *Mola* sp. A and *Mola* B morphology: **head bump/chin bump**: Yoshita et al. (2009), Matsuura (2015: fig. 6), Sawai et al. (2015), Ahuir-Baraja et al. (2017), Sawai et al. (2017); **protruding snout**: Fraser-Brunner (1951), E Sawai and M Nyegaard (unpublished data); **dorso- and ventrolateral ridges**: Thys et al. (2013: fig. 1B), Matsuura (2015: fig. 6), E Sawai and M Nyegaard (unpublished data), **smooth band**: Yoshita et al. (2009), Sawai et al. (2015), Ahuir-Baraja et al. (2017); **smooth band back-folds**: M Nyegaard and E Sawai (unpublished data); **clavus fin ray/ossicle counts**: Yoshita et al. (2009), Sawai (2016a), Sawai et al. (2017); clavus shape: Yoshita et al. (2009), Matsuura (2015: fig. 6), Ahuir-Baraja et al. (2017: fig. 1), Sawai et al. (2017); **skin structure**: Gaulide (1992), Sawai et al. (2015), Sawai (2016b).

2.4.4 Review of nominal Mola species

To confirm whether a scientific name consistent with the *M. tecta* characteristics was proposed in the past, we reviewed the original descriptions of nominal *Mola* species and other relevant names (Table 2.2), as well as associated historical literature. Due to the current uncertainty in regards to linking the genetic clades of *Mola* sp. A and *Mola* sp. B with established nomenclature, we did not attempt to assign species names to the nominal species descriptions, instead, we focused on establishing whether any nominal species description matched *M. tecta*. In support of this evaluation, early Molidae taxonomic history was also reviewed.

The original description of *M. mola* (*T. mola*, type locality in the Mediterranean) was brief, and included references to sunfish descriptions by Artedi (1738: 61, 83), Bianchi (1746: 297) and Gronovius (1754: 55), who in turn referenced various early, often nebulous, sunfish descriptions. Most of these were from the Mediterranean and the European seas, some describing and illustrating what we now recognize as '*Mola* forms' (e.g. Rondelet 1554: 424; Gessner 1560: 158, 159; Aldrovandi 1613: 412), others '*Ranzania* forms' [e.g. Aldrovandi 1613: 413; Jonstonus 1657: Pl. 9 (No. 1)]. As Linnaeus referenced both forms, it is not clear which sunfish he intended to describe. Although there is no known extant holotype for *M. mola* (*sensu* Parenti 2003), we know this species today from the historical application of the name. Linnaeus' *Mola*/*Ranzania* ambiguity was noted by several authors (e.g. Retzius 1785; Jacob 1826; Steenstrup & Lütken 1898) and caused confusion in early sunfish systematics; however, the name eventually came to mean *M. mola* as described in Fraser-Brunner (1951).

Early sunfish systematics was also influenced by differing opinions on how to resolve the taxonomy, in particular for the comparatively minute pre-juveniles, the elongated '*Ranzania*' shapes, and the many different sizes and shapes of *Mola* specimens. The collective endeavours of many naturalists resulted in numerous proposed classifications, as well as an extensive web of references and synonymies, with several nominal names based on re-grouping of existing species (Table 2.2). The distinct pre-juveniles were initially viewed as a separate species, or genus, resulting in a number of nominal species names, all later synonymized with *M. mola* (Fraser-Brunner 1951; Parenti 2003; Eschmeyer et al. 2017). In our view, the current lack of clarity on *Mola* spp. pre-juvenile morphology and identification precludes a conclusive assignment below genus level. However, these nominal species are improbable candidates for *M. tecta* due to their type localities in the Mediterranean, USA and 'tropical seas' (Table 2.2). While a small number of pre-juvenile specimen localities are not known, they were unlikely to originate from the temperate Southern Hemisphere, as this region was still relatively unexplored by science at the time they were reported (Table 2.2).

Most of the nominal species names were based on larger 'typical' sunfish specimens, with type localities in the Mediterranean and European seas. *Mola mola* was, until comparatively recently, considered the only *Mola* species present here; nevertheless, two records of *Mola* sp. A in the Mediterranean (Ahuir-Baraja et al. 2017; fig. 3) and one record of *M. tecta* from the Dutch coast (see below under Broader Molidae literature) preclude automatic assignment of all the European ocean sunfish names to *M. mola*. However, a careful review of the nominal species descriptions, illustrations and associated literature strongly suggests that *M. tecta* was not among them (Table 2.2). Of the nominal species in Table 2.2, three had type localities in the Southern Hemisphere, in areas we believe to be within the core distribution of *M. tecta*, and warrant further discussion.

Aledon capensis Castelnau, 1861 was described based on a 100 cm TL specimen caught in Table Bay near Cape Town, South Africa, where one of the *M. tecta* specimens in Bass et al. (2005) was also sampled (Figure 2.10). However, *A. capensis* was '*…très-remarquable par une avance en forme de nez, qui se prolonge au-dessus de la bouche*' […remarkable for a nose shape, extending beyond the mouth], and together with the description of a lobed clavus we conclude in agreement with Fraser-Brunner (1951), Parenti (2003) and Eschmeyer et al. (2017) that this specimen was *M. mola* (Table 2.2).

Mola ramsayi (Giglioli 1883) (originally *Orthragoriscus ramsayi*) was based on a large specimen [244 cm TL according to Whitley (1931)] from New South Wales in Australia, exhibited at the 1883 International Fisheries Exhibition in the UK. Giglioli (1883) examined it and concluded '*…it differs from our O. mola [=M. mola], [and] belongs to the southern hemisphere*' but gave few taxonomic details. The specimen became the name bearing type at the London Natural History Museum (BMNH 1883.11.29.22). As the Yoshita et al. (2009) *Mola* sp. C specimen was sampled at the type locality of *M. ramsayi* in New South Wales, and due to the unresolved discrepancy between the morphology of *Mola* sp. A and *M. ramsayi*, the question arises whether *Mola* sp. C could be *M. ramsayi*, and *Mola* sp. A another species altogether? It is an unlikely scenario however; photos of the newly restored *M. ramsayi* holotype (Natural History Museum, London: *www.instagram.com/p/BIzgLphgufI/*) clearly show a head bump and chin bump, a lack of, or inconspicuous, smooth band, all inconsistent with the head profile and prominent smooth band of *M. tecta*.

Orthagoriscus eurypterus Philippi, 1892 was described as a new species based on a large specimen (222 cm TL, 247 cm TBD), caught on 29 October 1889 off Chanaral in Chile. It was prepared and mounted prior to assessment, which likely rendered it somewhat distorted (Schneider 1930). The features, which made Philippi believe he had a new species, were '…*su forma mas alargada*' [the more elongated shape]; '…*la situacion de las aletas dorsal i anal, mucho ménos atrasada; la dorsal principia casi en la mitad de la lonjitud del cuerpo*' [the position of the dorsal and anal fins with the dorsal fin sitting almost in the middle of the body]; '…*la forma mui ancha de ellas*' [the wideness of the fins], and the edge of the clavus, described as '… *bien redondeado*' [well rounded] and '…*no ofrece una séria de osificaciones cortantes I separadas, parece mas bien todo osificado*' [not showing a string of individual ossifications but seeming to be ossified in the entire length]. While the TBD:TL ratio of 111% and the wide dorsal and anal fins are consistent with all three *Mola* species (Yoshita et al. 2009; this study), the combination of a rounded clavus and an elongated body on such a large fish point to it possibly being *M. tecta*. The position of the dorsal and anal fins 'almost in the middle of the body' is inconsistent with *M. tecta*, but could be an artefact of preparation and mounting. The ossification of the entire length of the clavus edge, however, is inconsistent with *M. tecta*, and we further note that while Philippi described the presence of a smooth band in detail, he did not make any mention of a back-fold. Phillipi's specimen is extant at the National Museum in Santiago de Chile (MNHNCH) as an unregistered specimen, which was not designated as holotype (A Castro, MNHNCH, pers comm November 2015). We examined this specimen via photographs provided to us by the museum, which confirmed distortion in the specimen and loss of over half the clavus edge (Figure 2.11A). However, the skin structure is well preserved, is densely populated with scales (Figure 2.11B), resembles the skin of a mounted *Mola* sp. B specimen described by Sawai et al. (2015: fig. 3B) and is dissimilar to that of *M. tecta* with its much wider set scales (Figure 2.9). A small stretch of the upper clavus edge of the *O. eurypterus* specimen is preserved (Figure 2.11A), and small ossicles are visible, interspersed with stretches of ossified clavus edge. This corresponds to the description by Philippi (1892) and is consistent with the clavus morphology of *M. mola*. We conclude, in line with Andersson's (1900) careful review of *M. mola* body dimensions, that *O. eurypterus* is '…*ett mycket stort exemplar af den vanliga* <u>*Orthagoriscus mola*</u>' [a very large specimen of the common *O. mola* (=*M. mola*)], and that the lack of clavus lobes was likely caused by damage during the preservation process. Our conclusion differs from Fraser-Brunner (1951), Parenti

(2003) and Eschmeyer et al. (2017), who list *O. eurypterus* as a junior synonym of *M. ramsayi* (Table 2.2).

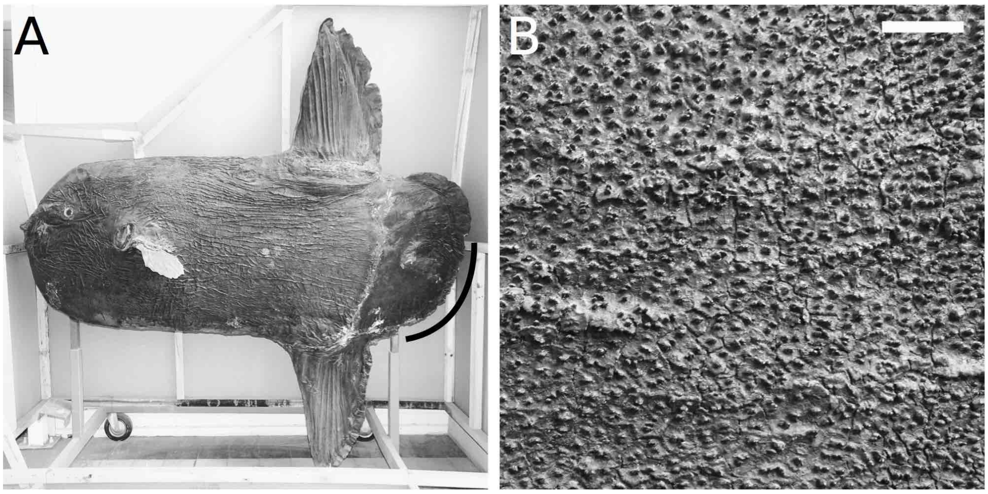


Figure 2.11 Presumed *Mola mola*: **A)** mounted skin of *Orthagoriscus eurypterus* (Philippi, 1892) extant at the National History Museum in Santiago de Chile (MNHNCH – unregistered, no type status). Black line indicates extent of clavus edge loss; **B)** macroscopic skin structure on the body (1 cm scale bar). Photographs by Augusto Tomás Cornejo Castro, MNHNCH.

2.4.5 Broader Molidae literature

Within the broader Molidae literature, we did not find any specimen descriptions matching *M. tecta*, with the exception of Lidth de Jeude (1890), who described and illustrated a '…*smooth band parting off backwards*' from the prominent smooth band on a 223-cm-long female sunfish, stranded on the Dutch coast in December 1889. Lidth de Jeude could not find mention of a back-fold elsewhere in the literature, discussed the difficulty of species identification and tentatively settled on *O. mola* (=*M. mola*). His illustration later appeared in Martin & Drewry (1978: fig. 171A) as the female form of *M. mola*, with a comment that the back-fold was probably atypical. Lidth de Jeude's detailed and to-scale drawing (Figure 2.12A) has a strong resemblance to *M. tecta*, and, while the TBD:TL ratio and count of eight ossicles are slightly higher than our findings, his skin structure description is consistent with *M. tecta*. The mounted skin is extant at the Leiden Naturalis Biodiversity Center in the Netherlands (RMNH.PISC.D.2059), and we examined this from photographs sent to us by the museum (Figure 2.12B–D); the body shape (Figure 2.12C), skin structure (Figure 2.12B) and smooth band with a backfold (Figure 2.12D) suggest it is likely *M. tecta*. Juede did not describe his specimen as a new species, and with no other indications of this species in the North East Atlantic, where sunfish sightings and strandings are not infrequent, it probably represents a rare occurrence of *M. tecta* outside the temperature waters of the Southern Hemisphere.

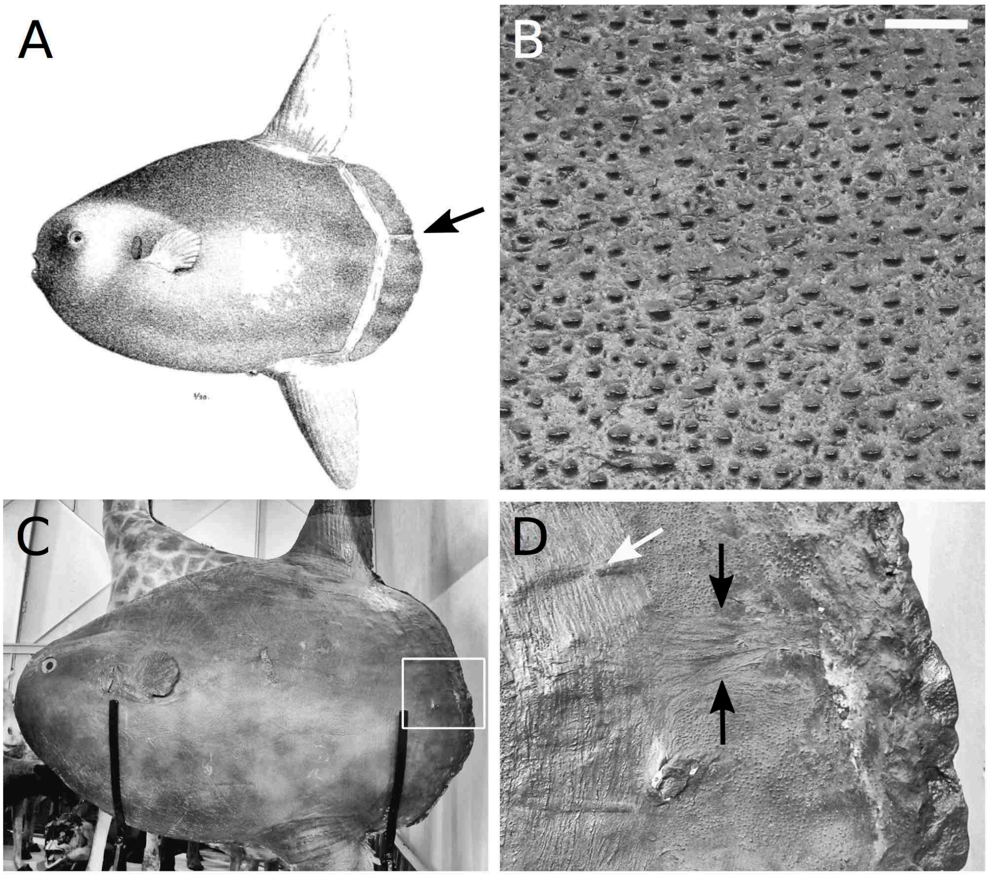


Figure 2.12 Presumed *Mola tecta*: **A)** Reproduction from Lidth de Jeude (1890) of 223 cm total length female sunfish, stranded on the Dutch coast in 1889, originally identified as *Orthragoriscus mola* (now *M. mola*), showing the smooth band back-fold (black arrow) observed by Lidth de Jeude; **B)** macroscopic skin structure on the body behind the pectoral fin; **C)** whole body view (white box indicates area of back-fold) and **D)** smooth band (white arrow) and back-fold (black arrows) on the mounted skin (RMNH.PISC.D.2059), extant at the Naturalis Biodiversity Center, The Netherlands. Photographs: Ronald de Ruiter, RMNH.

2.5 Conclusion

Our genetic and morphological analyses support the preliminary findings of Yoshita et al. (2009), that *Mola* sp. C is a species distinct from *Mola* sp. A and *Mola* sp. B. We have reviewed the relevant taxonomic literature and conclude that this is a new sunfish species not previously described. Our analysis and review also show that the CO1 locus, used as the standard DNA barcoding for many taxon, can, like the D-loop, be used to identify individuals of the three *Mola* species.

It seems baffling that such a large fish has gone unnoticed by the scientific community despite the extensive interest in sunfish taxonomy following, and even predating the time of Linnaeus. In addition to the difficulty of studying these fish due to their large adult sizes, the explanation may partly lie in that early research efforts were concerned predominantly with European and American sunfish, and that only three of the many nominal *Mola* species names (Table 2.2) were based on specimens from the Southern Hemisphere (i.e. Castalneu 1861; Giglioli 1883; Philippi 1892). By the time sunfish were recorded by the scientific community in Australia in the 1860s (e.g. Royal Society of Tasmania 1869) and 1870s in New Zealand (e.g. Hutton 1872, 1873), the Molidae literature was already extensive and complicated, with a general consensus emerging that most of the proposed *Mola* species were probably the same (i.e. Nardo 1840; Steenstrup & Lütken 1898). Later, even *M. ramsayi* did not always gain support as a separate species, despite surviving the Fraser-Brunner (1951) review as a 'Southern Hemisphere species'. McCann (1961), for example, summarized and discussed all New Zealand sunfish stranding records as *M. mola*, without any mention of *M. ramsayi*. In recent years, it has become generally accepted that both *M. mola* and *M. ramsayi* occur around New Zealand and southern Australia (e.g. Gauldie 1992; Hutchins 2001; Bray 2008; Swainston 2011; Stewart & Struthers 2015). Preliminary results from our Molidae biopsy program in Australia and New Zealand suggests that *Mola* sp. B (=presumably *M. mola*) is a relatively rare visitor in those waters, whereas *M. tecta* is relatively common (M Nyegaard, unpublished data). With few local *M. mola* for comparison, and in light of the long standing taxonomic confusion, *M. tecta* in this region has presumably been mistaken for *M. mola* and/or *M. ramsayi*, allowing it to 'hide in broad daylight'. Its detection constitutes the first proposed addition to the genus in 125 years since Philippi (1892), and 130 years since the last valid *Mola* species, *M. ramsayi* (Giglioli, 1883), was described.

The description herein of *M. tecta* sp. nov. clearly shows it as a separate species of *Mola*, and also highlights areas of further research to establish the degree of natural variation across its growth spectrum, geographical areas and between the sexes, to provide sufficient information for a comprehensive *Mola* identification key to be developed. The taxonomic review also highlights the need to formally link other genetic *Mola* spp. clades with nomenclature, and further describe their morphologies across the large size spectra. This would enhance our ability to differentiate the *Mola* species and thereby better understand their zoogeographies, an important basis for gauging vulnerability to bycatch in fisheries – a potential key threatening process to *M. mola* populations, at least locally (Liu et al. 2015). Like the other two *Mola* species, the eggs, pre-juveniles and juveniles of *M. tecta* need to be located and verified through molecular methods to describe all life stages. Our results to date are, however, sufficient to conclude that *M. tecta* should be considered a valid species in the genus *Mola*.

Table 2.1 *Mola tecta* sp. nov. holotype (in bold), paratypes and other material examined as part of this study.

Specimen	TL (cm)	Data source	Front profile	Clavus shape	Smooth band back-fold	Pectoral fin rays	Dorsal/ anal fin rays	Principal clavus fin rays[a]	Minor clavus fin rays	Clavus ossicles	Paraxial ossicles[a]	Scale shape
NMNZ P.005890	49.9	M:m,x	Tapered	Rounded, indent	Present (faint)	12	17/17	15 (7/8)	2	0	Separate	Rounded
AIM MA29864	51.1	M:m,x,	Tapered	Rounded, faint indent	Present (faint)	13	19/18	13 (6/7)	2	3 (1/2)	Separate	Rounded
NMNZ P.033995	57.2	M:m,x	Tapered	Rounded, indent	Present	12	18/17	14 (6/8)	2	2 (1/1)	Separate	Rounded
NMNZ P.001418	60.2	M:m,x	Tapered	Rounded, faint indent	Not visible	12	18/17	13 (5/8)	2	2 (0/2)	Separate	Rounded
G06	62	F:g,p	-	Rounded, indent	Present		-	-	-	-	-	-
NMNZ P.002980	64.6	M:m	Tapered	Rounded, indent	Present (faint)	11	18/17	-	-	3 (1/2)	Separate	Rounded
NZ18	65	F:g,m,d	Tapered	Rounded, wavy indent	Present	-	-	14 (7/7)	2	6 (3/3)	Separate	Rounded
NMNZ P.006126	64.8	M:m,x	Tapered	Rounded, indent	Present	11	18/17	15 (7/8)	2	5 (2/3)	Separate	Rounded
NZ19	69	F:g,m,d	Tapered	Rounded, indent	Present[b]	-	-	19 (9/10)[b]	3-4[b]	8 (3/5)[b]	Separate	Rounded
NMNZ P.034217	69.8	M:m,x	Tapered	Rounded, indent	Present	13	18/16	15 (7/8)	2	7 (3/4)	Separate	Rounded
OMNZ VT3248	78	C:m	Tapered	Rounded, indent	Present	-	-	-	-	-	-	Rounded
NMV A26565-001	78.5	F:p; M:m	Tapered	Rounded, indent	Present	-	-	15 (7/8)	-	6 (3/3)	Separate	Rounded
NMNZ P.034187	79.7	M:m,x	Tapered	Rounded, indent	Present	12	19/18	13 (6/7)	2	5 (2/3)	Separate	Rounded
NZ17	81	F:g,m	Tapered	Rounded, indent	Present	-	-	14 (6/8)	2	5 (2/3)	Separate	Rounded
SAMA F7542	Ca. 90	M:g,p	Tapered	Rounded, indent	Present	-	-	-	-	-	-	Rounded
NMV A18725	90.5	M:m	Tapered	Rounded, indent	Present	-	-	-	-	6 (2/4)	Separate	Rounded
NZ07	100	F:g,p	Tapered	Rounded, indent	Present	-	-	-	-	-	-	-

Specimen	TL (cm)	Data source	Front profile	Clavus shape	Smooth band back-fold	Pectoral fin rays	Dorsal/ anal fin rays	Principal clavus fin rays[a]	Minor clavus fin rays	Clavus ossicles	Paraxial ossicles[a]	Scale shape
NMNZ P.057679	**101.1**	**F:g,m,x; M:m**	**Tapered**	**Rounded, indent**	**Present**	**11**	**18/17**	**15 (7/8)**	**2**	**7 (3/4)**	**Separate**	**Rounded**
AIM MA30933	102	C:m,p	Tapered	Rounded, no indent[c]	Present	-	-	-	-	-	-	-
TMAG D3912	150	F:g,p	-	Rounded, indent	Present	-	-	-	-	-	-	-
NZ08	151	F:g,p	Tapered	Rounded, indent	Present	-	-	-	-	-	-	-
NZ12	155	F:g,m	Tapered	Rounded, indent	Present	12	-	13 (6/7)	2	6 (2/4)	Separate	Rounded
OMNZ X2017.19	169	F:g,m,p,d	Tapered	Rounded, indent	Present	-	-	-	-	-	-	Rounded
NZ14	170	F:g,p	-	Rounded, indent	Present	-	-	-	-	-	-	-
NZ09	193	F:g,p	Tapered	Rounded, indent	Present	-	-	-	-	-	-	-
NZ01	212	F:g,m,p	Tapered	Rounded, indent	Present	11	-	-	-	-	-	-
OMNZ VT3249	242	C:m	Tapered	Rounded, indent	Present	12	-	-	-	-	-	Rounded

All specimens: head bump absent; chin bump absent; snout absent; short dorso-lateral ridge present.

C: cast from fresh specimen; F: fresh specimen; M: museum specimen; P: specimen examined from photographs; TL: total length; d: digestive tract content; g: genetic sample; m: morphometric measurements; x: x-ray.

[a] Dorsally/ventrally of the smooth band back-fold.

[b] Abnormal clavus, see text for details.

[c] Indent likely lost during cast preparation.

Table 2.2 Nominal species listed as *Mola* sp. or nomen nudum by Fraser-Brunner (1951) (F), Parenti (2003) (P) and Eschmeyer et al. (2017) (E), with assessment against the *Mola tecta* sp. nov. morphological characteristics described in the text.

Assessment of nominal species against the *Mola tecta* morphological characteristics	**Species identity by taxonomic authorities**
***Tetraodon mola* Linnaeus 1758: 334. Type locality: Mediterraneo [Mediterranean Sea]. No types known.** Valid species, no citation. Literature source [Artedi 1738: 61, 83; Bianchi 1746: 297; Gronovius 1754: 55 (No. 125)]. Bianchi (1746) and Gronovius (1754) indicated *Ranzania laevis*, but Artedi (1738) indicated *Mola* sp. Identification through use of name (see text).	*M. mola* (F,P,E)
***Mola aculeata* Koelreuter 1766: 337, Pl. 8 (figs 2, 3). Type locality: not known. No types known.** Original source: two pre-juvenile specimens from the collection at the Imperial Academy of Sciences in St. Petersburg. Improbable candidate for *M. tecta* as specimens were highly unlikely to be from the temperature Southern Hemisphere due to the limited exploration of these waters at the time of reporting. Attempts to find the specimens as part of this study were unsuccessful.	*M. mola* (F,P,E)
***Diodon mola* Pallas 1770: 39, Pl. 4 (fig. 7). Type locality: Guinea. No types known.** Original source. Unneeded new name (Eschmeyer et al. 2017). Pre-juvenile; unlikely candidate for *M. tecta* due to tropical type locality.	*M. mola* (F,P,E)
***Ostracion mola* Forsskål 1775: xviii. Type locality: Malta.** Original source. Name only, not available (Eschmeyer et al. 2017).	Nomen nudum (P,E)
***Diodon nummularis* Walbaum 1792: 600. Type locality: not known. No types known.** Literature source [Ruysch 1710: 26 (No. 63), Pl. 3 (fig. 7); Koelreuter 1766: 337, Pl. 8 (figs 2, 3); Pallas 1770: 39, Pl. 4 (fig. 7); Gmelin 1788: 1452[a] (No. 3)]. Ruysch's (1710) description was superficial, based on a pre-juvenile specimen presumably from Indian waters (given as the old Dutch '*Indiaanisch*' and Latin '*Indicus*'). Gmelin (1788) was based on literature sources, including Pallas [1770: 39, Pl. 4 (fig. 7)], and Gronovius [1754: 55 (No. 125)]. The latter was based on literature sources Artedi (1738: 61) and Jonstonus [1657: Pl. 9 (No. 1)], both describing adult forms of *Mola* sp. and *R. laevis*, respectively. *Diodon nummularis* is an unlikely candidate for *M. tecta* due to the localities, and confusion of literature sources.	*M. mola* (P,E)
***Mola rotunda* Cuvier 1798: 324. Type locality: France. No types known.** Presumably literature source (unknown). Unneeded new name for *T. mola* Linnaeus, 1758 (Eschmeyer et al. 2017).	*M. mola* (F,P,E)
***Orthragoriscus fasciatus* Bloch & Schneider 1801: 511. Type locality: mari septentrionali ['northern ocean'; presumably the North Sea]. No types known.** Literature source (Duhamel du Monceau 1777: 306, Pl. 23). Illustration apparently of a specimen from Angola, Africa, but presumably sufficiently similar to include as illustration of *O. fasciatus*. Unlikely candidate for *M. tecta* due to description and illustration of protruding snout.	*M. mola* (F,P,E)
***Orthragoriscus hispidus* Bloch & Schneider 1801: 511. Type locality: not known. No types known.** Literature source [Pallas 1770: 39, Pl. 4 (fig. 7); Gmelin 1788: 1452]. Pre-juvenile; unlikely candidate (see *D. nummularis*).	*M. mola* (F,P,E)
***Cephalus brevis* Shaw 1804: 437, Pl. 175 (top). Type locality: European seas. No types known**. Literature source (Willughby 1686: 151, Pl. I.26; Linnaeus 1758: 334; Pennant 1776: 131; Bloch 1785: 75, Pl. 125). Unneeded new name based on previously described species and literature sources (Eschmeyer et al. 2017). Unlikely candidate for *M. tecta* due to mention of commonness in the Mediterranean (Bloch 1785; Pennant 1776).	*M. mola* (F,P,E)
***Cephalus pallasianus* Shaw 1804: 440. Type locality: tropical seas. No types known.** Literature source [Pallas 1770: 39, Pl. 4 (fig. 7); Gmelin 1788: 1452]. Prejuvenile, unlikely candidate for *M. tecta* (see *D. nummularis*).	*M. mola* (F,P,E)

Assessment of nominal species against the *Mola tecta* morphological characteristics	**Species identity by taxonomic authorities**
***Orthragus luna* Rafinesque 1810a: 17. Type locality: Sicilia [Sicily, i.e. Mediterranean Sea]. No types known**. Literature source (Linnaeus 1758: 334). We consider this an unneeded new name for *T. mola* Linnaeus 1758.	*M. mola* (F,P,E)
***Orthragus commersoni* Rafinesque 1810a: 17. Type locality: not known.** Unknown source. Name only, unavailable (Eschmeyer et al. 2017). Unlikely candidate for *M. tecta* due to context of Rafinesque (1810a) indicating *R. laevis*.	*R. laevis* (F), *nomen nudum* (P,E)
***Diplanchias nasus* Rafinesque 1810a: 17. Type locality: Sicilia [Sicily, i.e. Mediterranean Sea]. No types known.** Original source. Also in Rafinesque (1810b: 40) as *Diplanchias mola* (Eschmeyer et al. 2017). Unlikely candidate for *M. tecta* due to locality and description of protruding snout.	*M. mola* (F,P,E)
***Mola aspera* Nardo 1827a: 26, 35 (No. 26). Type locality: Adriatica [Adriatic Sea]. No types known**. Literature source [Gronovius 1763: 50 (No. 186); Bloch 1786: 83, Pl. 128; Daubenton 1787: 240 ('LUNE')]. Fraser-Brunner (1951: 115) and Eschmeyer et al. (2017) treated this as a questionably synonym. Insufficient taxonomic characters for assessment, however unlikely candidate for *M. tecta* due to locality.	?*M. mola* (F), *M. mola* (P,E)
***Mola hispida* Nardo 1827b: 104. Type locality: Adriatico mari [Adriatic Sea]. No types known.** Literature source [Koelreuter 1766: 337[b], Pl. 8[c] (figs 2, 3); Pallas 1770: 39, Pl. 4 (fig. 7[d]); Gmelin 1788: 1452]. Subjectively invalid, secondarily preoccupied in *Mola* by *O. hispidus* Bloch & Schneider 1801 (Eschmeyer et al. 2017). Pre-juvenile, unlikely candidate for *M. tecta* (see *D. nummularis*).	*M. mola* (F,P,E)
***Cephalus ortagoriscus* Risso 1826: 173. Type locality: Nice, France, i.e. Mediterranean Sea. No types known.** Original source (Risso 1810: 60) and literature source[e] (Rondelet 1558: 326; Bloch 1786: 83, Pl. 128; Cuvier 1817: 148). Unlikely candidate for *M. tecta* due to the number of clavus fin rays (18), its commonness in the Mediterranean, and the mention of sexual dimorphism.	*M. mola* (F,P,E)
***Diodon carinatus* Mitchill 1828: 264, Pl. 5 (fig. 1). Type locality: Massachusetts, USA. No types known.** Original source. Pre-juvenile, unlikely candidate for *M. tecta* due to type locality.	*M. mola* (F,P,E)
***Pedalion gigas* Guilding in Swainson 1838: 199, fig. 33. Type locality: West Indies. No types known.** Original source. Unlikely candidate for *M. tecta* due to wavy clavus on illustration.	*M. mola* (F,P,E)
***Molacanthus pallasii* Swainson 1839: 329. Type locality: not known [given as Guinea in Pallas, 1770]. No types known.** Literature source [Pallas 1770: 39, Pl. 4 (fig. 7)]. We consider this an unneeded new name based on previously described species. Pre-juvenile, unlikely candidate for *M. tecta* (see *D. nummularis*).	*M. mola* (F,P,E)
***Tympanomium planci* Ranzani 1839: 74, 76, table. Type locality: mari adriatico [Adriatic Sea]. Holotype (unique): whereabouts unknown.** Literature source (Bianchi 1755: 331). Subjectively invalid, preoccupied in *Mola* by *M. planci* Nardo (1827a) (Eschmeyer et al. 2017). Unlikely candidate for *M. tecta* due to protruding snout and scalloped clavus.	*M. mola* (F,P,E)
***Trematopsis willugbei* Ranzani 1839: 74, table. Type locality: oceano ['ocean'] [given as Mediterraneo, atque etiam in Oceano (Mediterranean and also in the Ocean) in Willughby 1686]. Holotype (unique): not found at MZUB.** Literature source (Willughby 1686: 151, Pl. I.26), based on original source. We consider the validity of the species doubtful, as it was based on four holes in a rectangle on top of the head (Willughby 1686), likely from external damage. Unlikely candidate for *M. tecta* due to locality, description of a rounded clavus without mention of an indentation, and a protruding snout on the included illustration by Salviani (1554: 154).	*M. mola* (F,P,E)
***Ozodura orsini* Ranzani 1839: 75, 80, table, Pl. 6 (right). Type locality: mari adriatico [Adriatic Sea]. Holotype (unique): mount on wall at MZUB.** Extant at MZUB, no registration number available. Original source. Unlikely candidate for *M. tecta* due to locality, description and illustration, in particular a rounded clavus without an indentation, and merged paraxial ossicles.	*M. mola* (F,P,E)

Assessment of nominal species against the *Mola tecta* morphological characteristics	**Species identity by taxonomic authorities**
***Orthragoriscus retzii* Ranzani 1839: 75, table. Type locality: not known [given as Landscrona (i.e Øresund between Denmark and Sweden) in Retzius, 1785]. No types known.** Literature source (Retzius 1785: 115, Pl. 4; Retzius 1800: 310[f]). Based on Retzius (1785) (Eschmeyer et al. 2017). Unlikely candidate for *M. tecta* due to location, rough skin and detailed description and illustration of a prominent smooth band without mention of a back-fold.	*M. mola* (F,P,E)
***Orthragoriscus ghini* Ranzani 1839: 75, table. Type locality: mari mediterraneo [Mediterranean Sea]. No types known.** Literature source (Salviani 1554: 154). Unlikely candidate for *M. tecta* due to protruding snout on illustration (Salviani 1554: 154).	*M. mola* (F,P,E)
***Orthragoriscus rondeletii* Ranzani 1839: 75, table. Type locality: mari mediterraneo [Mediterranean Sea]. No types known.** Literature source (Rondelet 1554: 424). Lack of taxonomic details precludes assessment, but unlikely candidate for *M. tecta* due to commonness at the locality.	*M. mola* (F,P,E)
***Orthragoriscus redi* Ranzani 1839: 75, table. Type locality: mari mediterraneo [Mediterranean Sea]. No types known.** Literature source (Redi 1684: 166). Vague description; lack of taxonomic details precludes assessment. Unlikely candidate for *M. tecta* due to commonness at the locality.	*M. mola* (F,P,E)
***Orthragoriscus alexandrini* Ranzani 1839: 75, 78, table, Pl. 6 (left). Type locality: mari adriatico [Adriatic Sea]. No known types.** Original source. Unlikely candidate for *M. tecta* due to description and illustration of large head bump and rounded clavus without indentation.	*M. mola* (F,P,E)
***Orthragoriscus blochii* Ranzani 1839: 76, table. Type locality: mari oceano ['ocean sea', possibly Atlantic]. No types known.** Literature source [Bloch 1786: 83, Pl. 128; Bonnaterre 1788: 54, Pl. 17 (fig. 54); Shaw 1804: 437, Pl. 175 (top)]. Unlikely candidate for *M. tecta* due to description of rough skin, and mention of commonness at the locality.	*M. mola* (F,P,E)
***Orthragoriscus elegans* Ranzani 1839: 76, table. Type locality: mari atlantico [Atlantic Sea]. No types known.** Literature source (Bloch & Schneider 1801: 511, Pl. 97). Parenti (2003: 3) and Eschmeyer et al. (2017) synonymized *O. elegans* with *M. mola*. However, *O. elegans* was based on *O. oblongus* in Bloch & Schneider (1801: 511, Pl. 97), which is considered a synonym of *R. laevis* by Parenti (2003: 4) and Eschmeyer et al. (2017). Unlikely candidate for *M. tecta* as the illustration in Bloch & Schneider (1801: Pl. 97) is clearly *R. laevis*.	*R. laevis* (F), *M. mola* (P,E)
***Pallasia pallasi* Nardo 1840: 112. Type locality: not known [given as Guinea in Pallas, 1770]. No types known.** Literature source [Pallas 1770: 39, Pl. 4 (fig. 7)]. *Pallasina pallasi* [sic] in Fraser-Brunner (1951: 110, 115), name deviation discussed in Parenti (2003: 6) and Eschmeyer et al. (2017). Unneeded new name based on previously described species. Pre-juvenile; unlikely candidate for *M. tecta* (see *D. nummerlaris*).	*M. mola* (F), nomen nudum (P,E)
***Orthragoriscus lunaris* Gronow in Gray 1854: 165. Type locality: Mediterranean Sea. No types known.** Literature source[g] [Boussuet, 1558: 179; Gronovius, 1763: 50 (No. 185); Linnaeus 1766: 412 (gen. 137, No 7); Bianchi 1755: 331; Borlase 1758: 268, Pl. 26 (fig. 7); Klein 1742: 23 (No. 31)]. This author probably meant to describe a *Mola* species but confused the characteristics and references of *R. laevis* and *Mola* sp., precluding assessment.	*R. laevis* (F), *M. mola* (P,E)
***Orthagoriscus analis* Ayres 1859: 31, fig. 5 (p. 54). Type locality: Santa Barbara Channel, California, USA. Holotype (unique): CAS (lost in 1906).** The holotype was unregistered, and lost along with all records in an earth quake and subsequent fire (D Catalina, California Academy of Sciences, pers comm, April 2017). Original source. Specimen with damaged dorsal and anal fins (Günther 1870: 319). Description rudimentary, illustration extremely crude, precludes identification below genus level. Unlikely candidate for *M. tecta* based on locality.	*M. mola* (F,P,E)
***Aledon storeri* Castelnau 1861: 76. Type locality: not known [given as Boston Bay, Massachusetts, USA. in Storer, 1839]. No types known.** Literature source [Storer 1839: 515, Pl. 3 (fig. 1)]. Storer (1839) based on original source. Unlikely candidate for *M. tecta* based on scalloped clavus and a protruding snout.	*M. mola* (F,P,E)

Assessment of nominal species against the *Mola tecta* morphological characteristics	**Species identity by taxonomic authorities**
***Pedalion capensis* Castelnau 1861: 75. Type locality: la baie dela Table [Table Bay, South Africa]. No types known.** Original source. Unlikely candidate for *M. tecta* due to description of rough skin, protruding snout and scalloped clavus.	*M. mola* (P,E)
***Aledon capensis* Castelnau 1861: 75. Type locality: la baie dela Table [Table Bay, South Africa].** New combination for *P. capensis* described by Castelnau in the same paper, placed into new genus *Aledon* at the end of the article (Eschmeyer et al., 2017); an unneeded new name.	*M. mola* (F,P)
***Orthragoriscus ramsayi* Giglioli 1883: 315. Type locality: New South Wales, Australia. Type BMNH 1883.11.29.22; stuffed skin, poor condition; restored in** 2017 (see text). Holotype listed as BMNH 1888.11.29.22 in Eschemeyer (2017). Original source. Unlikely candidate for *M. tecta* (see text).	*M. ramsayi* (F,P,E)
***Orthagoriscus eurypterus* Philippi 1892: 14, Pl. 6 (fig. 1). Type locality: Chañaral, Chile. Mounted skin at MNHNCH, unregistered, not designation as holotype (see text).** Original source. Unlikely candidate for *M. tecta* (see text).	?*M. ramsayi* (F), *M. ramsayi* (P,E)

'Original source' means the species description was based on a specimen; 'literature source' means the species description was based on existing literature. Type status from Eschmeyer et al. (2017) unless otherwise stated. Museum codes according to Sabaj (2016).

[a] Page 1450 in Walbaum (1792), presumably a typographical error.

[b] Page 440 in Nardo (1827b); [c] Pl. 6 in Nardo (1827b); [d] fig. 9 in Nardo (1827b) presumably typographical errors.

[e] We could not resolve referenced work: 'Salv., 55' (possibly Salviani, 1554: 154); 'Will., 16.' (possibly Willughby, 1686: 151. Pl. I.26); 'Lac., 2,364' (possibly Lacépède, 1798: 633).

[f] Page 320 in Ranzani (1839): table.

[g] We could not resolve referenced work: 'Charlet, Onom. Zoic. Pisc. P.9 $4' [possibly Charleton, 1668: 129 (No. 3)].

Appendix A2.1 Supplementary material to Chapter 2

Table A2.1.1 National Center for Biotechnology Information(NCBI) Accession numbers for mtDNA D-loop and cytochrome *c* oxidase 1 (CO1) sequences analysed as part of this study. Institutional codes according to Sabaj (2016), except ABTC (Australian Biological Tissue Collection) and OMNZ (Otago Museum).

Specimen	**Species**	**Accession number (D-loop)**	**Accession number (CO1)**
G06	*Mola tecta* sp. nov.	MF158131	MF158115
G10	*Mola* sp. A[a]	MF158132	MF158116
H04	*Mola* sp. A[a]	MF158133	MF158117
NMNZ P.056071[b]	*Mola* sp. A[a]	MF158140	MF158118
NMNZ P.057679[c]	*M. tecta* sp. nov.	MF158147	MF158119
NZ01	*M. tecta* sp. nov.	MF158134	n/a
NZ07	*M. tecta* sp. nov.	MF158137	MF158120
NZ08	*M. tecta* sp. nov.	MF158138	MF158121
NZ09	*M. tecta* sp. nov.	MF158139	MF158122
NZ12	*M. tecta* sp. nov.	MF158141	MF158123
NZ14	*M. tecta* sp. nov.	MF158142	MF158124
NZ16	*Mola* sp. B (*M. mola*)	MF158143	MF158125
NZ17	*M. tecta* sp. nov.	MF158144	MF158126
NZ18	*M. tecta* sp. nov.	MF158145	MF158127
NZ19	*M. tecta* sp. nov.	MF158146	MF158128
OMNZ X2017.18	*Mola* sp. A[a]	MF158135	MF158129
OMNZ X2017.19	*M. tecta* sp. nov.	MF158136	MF158130
SAMA F7542[d]	*M. tecta* sp. nov.	MF158148	n/a
TMAG D3912	*M. tecta* sp. nov.	MF158149	n/a

[a] Presumably *M. ramsayi* (Giglioli, 1883)

Tissue sample registration numbers: [b] NMNZ P.056071/TS1; [c] NMNZ P.057679/TS1; [d] ABTC 21528.

n/a: not available.

Table A2.1.2 Whole or partial Molidae cytochrome *c* oxidase1 (CO1) sequences lodged with Barcode of Life Data Systems (BOLD) and/or the National Center for Biotechnology Information, as of 19 March 2017.

Registered taxon	Identification (this study)	Accession number	BOLD sequence number
Masturus lanceolatus	*Ma. lanceolatus*	-	I.44396-001
Ma. lanceolatus	*Ma. lanceolatus*	AP006239[a]	-
Ma. lanceolatus	*Ma. lanceolatus*	KF930108	UKFBI660-08
Ma. lanceolatus	*Ma. lanceolatus*	KR261939	-
Ma. lanceolatus	*Mola* sp. A[b]	KU945271	-
Ma. lanceolatus	*Ma. lanceolatus*	KC576974	GBGCA2306-13
Mola mola	*Mola* sp. A[b]	AP006238[a]	-
M. mola	*Mola* sp. A[b]	HQ167728	ANGBF1325-12
M. mola	*Mola* sp. B[c]	JF952793	ABFJ213-07
M. mola	*Mola* sp. B[c]	JQ775087	FCFPW216-06
M. mola	*Mola* sp. B[c]	JQ775088	FCFPW158-06
M. mola	*Mola* sp. B[c]	JX438518	TCHE024-12
M. mola	*Mola* sp. B[c]	KF025665	GBGCA4902-13
M. mola	*Mola* sp. B[c]	KF737069	-
M. mola	*Mola* sp. B[c]	KF737070	-
M. mola	*Mola* sp. B[c]	KJ128550	GBGCA8530-15
M. mola	*Mola* sp. B[c]	KJ128551	GBGCA7468-15
Ranzania laevis	*R. laevis*	KJ768291	MLFPI229-11
R. laevis	*R. laevis*	-	NC_007887
R. laevis	*R. laevis*	-	BIM492-16
R. laevis	*R. laevis*	-	TZSAL370-13
R. laevis	*R. laevis*	-	TZSAL764-13
R. laevis	*R. laevis*	-	TZSAL805-13
R. laevis	*R. laevis*	AP006047[a]	-
R. laevis	*R. laevis*	DQ521011	GBGC1432-06
R. laevis	*R. laevis*	HQ167729	ANGBF1326-12
R. laevis	*R. laevis*	HQ945963	DSFSG385-10
R. laevis	*R. laevis*	KF025666	GBGCA4901-13
R. laevis	*R. laevis*	KF027509	-
Tetradontiformes	*Mola* sp. B[c]	-	FMVIC396-08
Tetradontiformes	*M. tecta* sp. nov.	-	AMS174-08
Tetradontiformes	*R. laevis*	-	MLIII460-08
Tetradontiformes	*R. laevis*	-	NEEL3292-14
Tetradontiformes	*R. laevis*	-	SAIAC523-09

[a] Whole mitogenomes

[b] Presumably *M. ramsayi* (Giglioli, 1883)

[c] Presumably *M. mola* (Linnaeus, 1758)

Table A2.1.3 Morphometric measurements of the *Mola tecta* sp. nov. holotype (NMNZ P.057679) after initial freezing and thawing (fresh state), and after preservation (1 month in formalin, followed by 1 month in an isopropyl alcohol stepping process).

Measurement	Fresh state (cm)	Preserved state (cm)
Total Length (TL)	101.1	101.2
Pre-Clavus Band Length (PCBL)	82.9	78.7
Pre-Anal Fin Length (PAFL)	65.1	60.4
Head Length (HL)	27.9	30.6
Snout Length (SnL)	11.4	13.7
Total Body Depth (TBD)	126.7	120.6
Body Depth (BD)	57.6	57.7
Pre-Pectoral Fin Depth (PPFD)	53.6	57.2
Head Depth (HD)	37.0	38.9

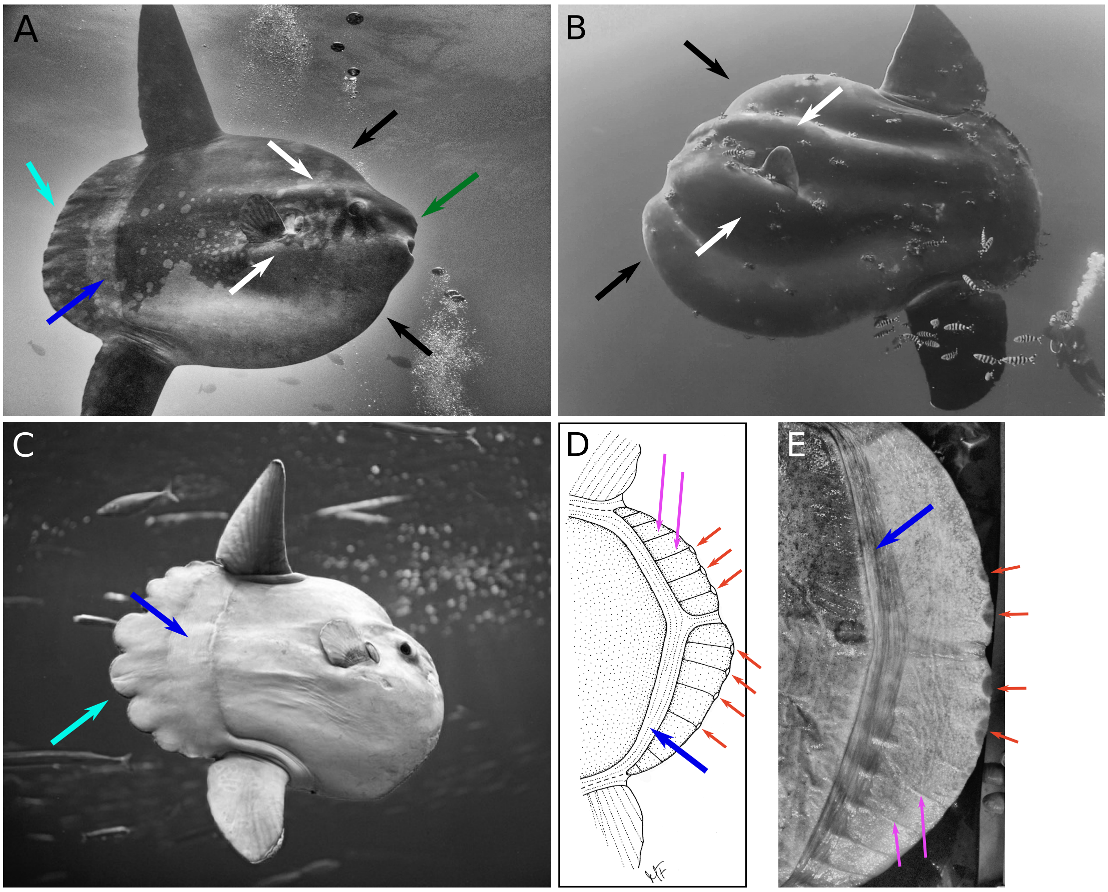


Figure A2.1.1 Examples of sunfish morphological traits: head bump and chin bump (black arrows), dorso-lateral and ventro-lateral ridges (white arrows), protruding snout (green arrow), smooth clavus band (blue arrows), smooth and lobed clavus edge (light green arrows) on **A)** and **C)**, respectively, ossicles (red arrows) and fin rays (pink arrows). Images by Cornelia Thieme (A), Eric van der Goot (B), Hirtshals Oceanarium (C) and illustrated by Michelle Freeborn (D). Reproduced with permission.

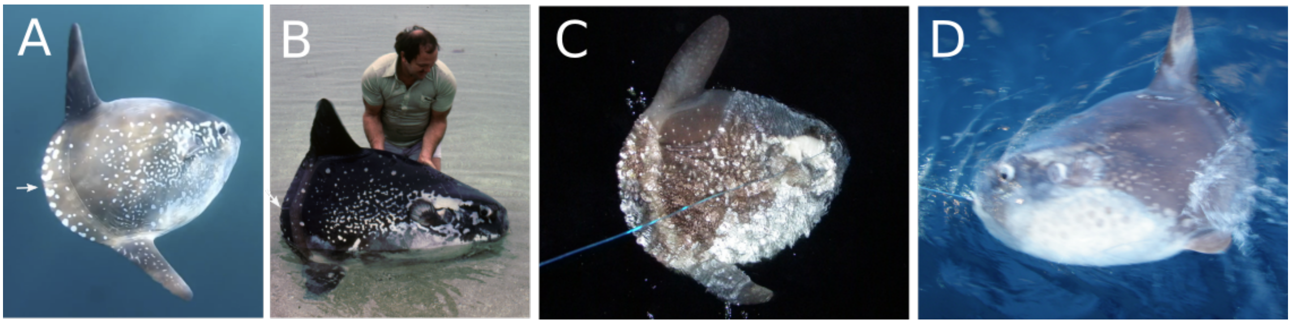


Figure A2.1.2 Colouration of live *Mola tecta* **A)** Swimming near the surface in Reserva Marina Isla Chañaral, Chile, 2015. Still from footage by César Villarroel (https://vimeo.com/129499857); **B)** stranded and re-floated in Port Phillip Bay, Victoria, 1979. Photograph by Martin Gomon, Museums Victoria, Australia; **C)** and **D)** caught and released alive from tuna longlines off New Zealand, July 2013. Photographed by fisheries observer Marli Dee, New Zealand Ministry of Primary Industries. A) and B) identified from morphology, including a smooth band back-fold (white arrows); C) and D) identified genetically (D-loop).

Chapter 3 Giant jelly eaters on the line: species distribution and bycatch of three dominant sunfishes in the Southwest Pacific

3.1 Abstract

The ocean sunfishes have a long and confusing taxonomic legacy, clouding the global zoogeography of each species and hindering fisheries bycatch assessments. The traditional view of *Mola mola* as the most common sunfish species worldwide is challenged by our findings from Australia and New Zealand, revealing that three large sunfishes, *Masturus lanceolatus*, *Mola alexandrini* and *Mola tecta*, dominate the tropical/subtropical, warm-temperate and cold-temperate waters here, respectively, while *Mola mola* – both Pacific and Atlantic clades – is relatively rare. These findings were based on phylogenetic (mtDNA D-loop) and/or morphological species identification of sunfish from longline bycatches (*n* = 106), natural history museum collections (*n* =45) and other sources (*n* = 12), informed by recent advances in the taxonomy of the genus *Mola*. Furthermore, separation in species distributions were seen when comparing sampling latitude and sea surface temperature. The findings imply that the longline fisheries observer sunfish data from Australia and New Zealand is a mix of species, and not dominated by *M. mola* as previously assumed. Mean catch per unit Effort (2001 – 13) in 1° latitude/longitude grids off Pacific Australia and New Zealand were predominantly < 1 sunfish.1,000 hooks^{-1} (up to 6.5 in some areas) with no statistical significant upwards or downwards trends detected over time in four fishing ground subareas, each presumably dominated by either *Masturus lanceolatus*, *Mola alexandrini* or *Mola tecta*. Widespread specimen identification errors had previously obscured a more complex Molidae zoogeography in the area, highlighting that phylogenetic analyses of sunfish bycatch globally would benefit species-level conservation status evaluations.

3.2 Introduction

The ocean sunfishes (genera *Mola* and *Masturus*, Family Molidae) are a somewhat overlooked group of large teleosts, famous for their odd shape resembling ‘swimming heads’ without tails, and for including the World’s heaviest bony fish (Sawai et al. 2018). They are difficult to study, but with the advances in modern technology they have experienced a surge in research in recent years, revealing they are not the peculiar, sluggish oddities of nature they have traditionally been considered (e.g. Cartamil and Lowe 2004; Watanabe and Sato 2008). Instead, their diet and evidently high numbers worldwide suggest they play an important ecological role as predators of gelatinous zooplankton (Breen et al. 2017; Grémillet et al.

2017; Phillips et al. 2017). Advances in telemetry, digital imagery and other technologies continue to confirm that ocean sunfish are active predators, hunting at depth in dynamic frontal systems (Nakamura et al. 2015; Thys et al. 2015; Sousa et al. 2016b). However much of their life history is still unknown, as is the global zoogeography of all taxa in the family.

Currently, five species in three genera are recognized in the Molidae, namely the small Slender sunfish *Ranzania laevis* (Pennant 1776), the Sharptail sunfish *Masturus lanceolatus* (Liénard 1840), the Ocean sunfish *Mola mola* (Linnaeus 1758), the Bump-head sunfish *Mola alexandrini* (Ranzani 1839) (Sawai et al. 2018), and the newly described Hoodwinker sunfish *Mola tecta* Nyegaard et al. 2017 (Eschmeyer et al. 2018; Froese and Pauly 2018). All five species obtain large adult sizes of > 2.4 m in total length (> 3 m for some species) (Gudger and MacDonald 1935; Nyegaard et al. 2018b [Chapter 2]; Sawai et al. 2018). The current Molidae taxonomy is a modification of Fraser-Brunner (1951), updated with recent insights from molecular and morphological studies. Briefly, the genetic clade *Mola* sp. A of Yoshita et al. (2009) was recently equated to *M. alexandrini*, a valid, senior synonym of *M. ramsayi* (Sawai et al. 2018), while *Mola* sp. C of Yoshita et al. (2009) was described as a new species, *M. tecta* (Nyegaard et al. 2018b [Chapter 2]). The third clade, *Mola* sp. B of Yoshita et al. (2009), has been equated to *M. mola* (Yoshita et al. 2009; Matsuura 2015; Sawai et al. 2018), with residual uncertainties in the nomenclature, as this species appears to include two basin-wide groups (Atlantic vs Pacific), as revealed by phylogenetic analysis (Bass et al. 2005; Ahuir-Baraja et al. 2017; Sawai et al. 2017). Here, the nomenclature follows Sawai et al. (2018) by treating *Mola* sp. B as a single species, *M. mola*, awaiting taxonomic resolution of the two genetic clades, which are herein referred to as Atlantic and Pacific *M. mola*, respectively. Within *M. alexandrini*, ‘subtropical’ and ‘temperate’ groups have been inferred from phylogenetic analysis (Yoshita et al. 2009; Ahuir-Baraja et al. 2017).

3.2.1 Distribution and interactions with fisheries

To date, the majority of ecological and biological research on the Molidae has been undertaken on *M. mola* in the northern hemisphere. Anecdotally, this is considered to be the most common of the large sunfish species globally, with a worldwide distribution in temperate and tropical waters (Pope et al. 2010; Liu et al. 2015; Froese and Pauly 2018). However, a legacy of taxonomic confusion (Nyegaard et al. 2018b [Chapter 2]; Sawai et al. 2018) has obscured a more complex zoogeographical pattern within the *Mola* species. The traditional view of *M. alexandrini* being restricted to the South Pacific, with sympatric occurrence with *M. mola* in Australian waters (Fraser-Brunner 1951), has been challenged in recent years, as molecular, morphological and ecological studies have confirmed that

M. alexandrini is found over a wide range in both hemispheres (Yoshita et al. 2009; Thys et al. 2013 2016; Ahuir-Baraja et al. 2017; Sawai et al. 2018). Prior to its recent description, *Mola tecta* was mistaken for *M. mola* or *M. ramsayi* (now *M. alexandrini*) and while *M. tecta* appears to have a circumpolar distribution in temperate waters of the southern hemisphere (Nyegaard et al. 2018b [Chapter 2]), little is known of this recently described species. *Masturus lanceolatus* is considered to be widely distributed throughout the tropics (Liu et al. 2015; Froese and Pauly 2018) but little has been published on this species, despite it being subjected to a targeted fishery in Taiwan (Liu et al. 2009).

Ocean sunfishes are caught as bycatch in various fisheries around the world and their bycatch is thought to be so significant that recently, *M. mola* was listed by the International Union for the Conservation of Nature (IUCN) as 'Vulnerable' globally. The concerns stem, in part, from the high level of estimated bycatch in the South African longline fishery (Sims et al. 2009b), and the possibility that longline bycatch elsewhere may be similarly high (Liu et al. 2015). Large total bycatches, as well as rapid declines in the total bycatch of *M. mola* in other types of fisheries were also identified as of concern. However, a paucity of analysis of long-term fisheries bycatch data in most parts of the world prevents a more detailed assessment (*op. cit.*). The other large sunfish species are either not considered of concern by the IUCN (*Ma. lanceolatus* is currently considered of 'Least Concern'), or are not assessed (*M. alexandrini* and *M. tecta*).

3.2.2 Sunfishes in Australia and New Zealand

Knowledge of the species identity of sunfish in Australian and New Zealand waters stems mainly from museum collections and information collected from fisheries. Ocean sunfishes are caught as bycatch in the Australian and New Zealand longline fisheries for tuna and billfish (Griggs and Baird 2013; Tuck et al. 2013), where data have been recorded for more than a decade. In Australia, they are predominantly recorded by fisheries observers as 'Unspecified Molidae' or *M. mola*, and evaluated by management as a mix of *M. mola* and *M. alexandrini* (Ward and Epe 2008; Tuck et al. 2013). Both *M. mola* and *M. alexandrini* are listed as "High Risk" bycatch species in the longline fishery off eastern Australia, with robust impact assessments hindered by a paucity of data on life history and post-release survival (Ward and Epe 2008; AFMA 2013, 2014). In New Zealand, all sunfish longline interactions are recorded and managed as *M. mola* (Francis et al. 1999; Griggs and Baird 2013; MPI 2016a). Similar broad sunfish bycatch categories, including the generic use of "*M. mola*", are also common in longline fisheries in other parts of the world (e.g. Petersen 2005; Fulling et al. 2007; Burgess et al. 2010; Domingo et al. 2012; Cambiè et al. 2013; Clarke et al. 2014).

Although four large Molidae species are currently listed from Australian waters (i.e. *Ma. lanceolatus*, *M. mola*, *M. alexandrini* and *M. tecta*; Hutchings 2001; Bray 2008; Fishes of Australia 2017), *M. mola* has long been considered the most common species here (e.g. Pope et al. 2010; Atlas of Living Australia 2017). In New Zealand, *M. mola* was traditionally listed as the only large sunfish species (Parrott 1960; McCann 1961; Doak 1972), with the later addition of *Ma. lanceolatus* (Paulin et al. 1982; Paul and Heath 1997; Paul 2000). A recent review of the Molidae in New Zealand, however, listed *Ma. lanceolatus* and *M. alexandrini*, while excluding *M. mola* (Roberts et al. 2015; Stewart and Struthers 2015). In recent years, phylogenetic analyses of a small number of sunfish samples from Australia and New Zealand have confirmed that all four large sunfish species are indeed present in the region, i.e. nesting in the *Ma. lanceolatus* (n = 1), *M. alexandrini* (n = 7), *M. tecta* (n = 14) and *M. mola* (n = 2) clades (Yoshita et al. 2009; Yamanoue and Sawai 2012; Nyegaard et al. 2018b [Chapter 2]), however the relative abundance and distribution of each species is not known.

The objectives of this study are to determine which of the large species of Molidae (i.e. excluding *R. laevis*) most commonly occur in Australia and New Zealand waters, whether the distribution patterns differ between species, if they are associated with different sea surface temperature regimes, and if longline bycatch data reveal a decreasing pattern over time. Sunfish skin samples were obtained from longline bycatches, museum collections and stranding events, and the species identity determined phylogenetically. A review was also undertaken of sunfish specimens held in museum collections in Australia and New Zealand, with the species confirmed morphologically, according to recent advances in the taxonomy of the *Mola* genus (Nyegaard et al. 2018b [Chapter 2]; Sawai et al. 2018). Taking all data sources into consideration, the distribution of each species was examined in relation to latitude and satellite sea surface temperature (SST). Based on the results, catch per unit effort (CPUE) was calculated from long-term fisheries observer longline bycatch data from Australia and New Zealand, as a proxy for sunfish abundance, to identify any decreasing trends within selected areas of the overall fishing grounds. Finally, overall CPUEs were compared with the rates of sunfish bycatch in the South African longline fishery.

3.3 Material and methods

3.3.1 Overview of the Australian and New Zealand longline fishery

The longline fishery in Australia is divided into the Eastern (ETBF) and Western Tuna and Billfish Fishery (WTBF) (Figure 3.1). Fishing within the ETBF occurs over a wide area, from north Queensland to Tasmania. The majority of fishing effort is concentrated relatively close to the coast, excluding the Great Barrier Reef Marine Park (Figure 3.1). A small number of vessels (< 3) currently participate in the WTBF (AFMA 2014), with most of the fishing effort occurring off southwest Western Australia (data not shown due to confidentiality agreements). An Australian Fisheries Management Authority (AFMA) domestic fisheries observer program was established in 2001, mainly covering the ETBF. In 2014, the program was transformed from observer based to video monitoring of the fishing vessels. In New Zealand, the tuna longline fishery targets a wide area, with the majority of effort concentrated along the northeast coast of the North Island, as well as an area west of the South Island, which is fished seasonally (mainly April – June) (Figure 3.1). The New Zealand observer program was established in 1985, with increased observer coverage around 2000 onwards. The program is currently managed by the New Zealand Ministry for Primary Industries Manatū Ahu Matua (MPI). In both the Australia and New Zealand longline fisheries, sunfish are predominantly released alive, and very rarely retained (AFMA 2011; MPI 2016b).

3.3.2 Tissue samples

Skin biopsies were obtained from sunfish caught as bycatch in the ETBF and New Zealand longline fisheries between February 2013 and February 2014, and between July 2013 and August 2014, respectively, by fisheries observers. Commercial crew members on two vessels in the WTBF, trained by an AFMA observer, biopsy sampled sunfish during April and May 2014, with one additional sample obtained in September 2016. In all cases, skin tissue was sampled with a 3 m-long pole, mounted with a biopsy head (0.5 mm diameter), and a "stopper" preventing the biopsy tip penetrating beyond 8 mm. The biopsy samples were recovered from the posterior body area of the fish while held alongside the vessel, prior to live release. The total fish length (TL) was estimated by eye by the sampler. In some cases, small sunfish were hauled onboard for disentanglement, and measured to the nearest cm with a measuring tape or measuring board. Fisheries observers assigned a species code to each specimen according to their standard protocol, while the commercial crew classified the clavus shape of the fish as with or without a pointed clavus extension ("central lobe" in Fraser-Brunner 1951), i.e. distinguishing between *Mola* spp. and *Ma. lanecolatus*. Each tissue

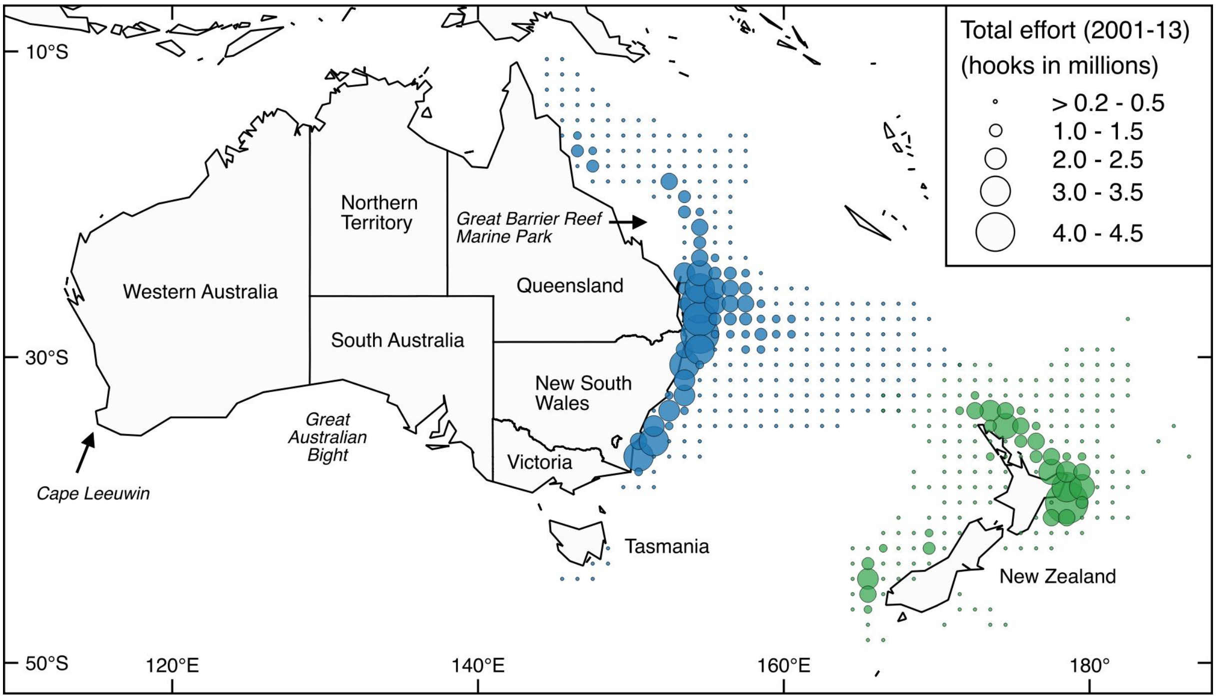


Figure 3.1 Total number of hooks deployed in 1° latitude/longitude squares in the Australian Eastern Tuna and Billfish Fishery (ETBF) (blue circles) and New Zealand longline fishery (green circles) between 2001-2013. Data supplied by the Australian Fisheries Management Authority and the New Zealand Ministry for Primary Industries.

sample was placed in a 2 mL vial with the preservative RNA*Later* and stored frozen on board the vessel, then sent to the laboratory at ambient temperature (< 1 week). All biopsy sampling by observers was opportunistic and undertaken as logistics, other duties and catch processing allowed. Sunfish of all species and sizes were targeted indiscriminately when circumstances permitted. Throughout the sampling period, the spatial and temporal fishing activities and observer coverage were influenced by external factors such as weather, occurrence of target species, bait availability, and so forth.

Since 2013, volunteers and natural history museums across Australia and New Zealand informed us of local sunfish strandings, which were attended by the authors when possible, or which were attended by volunteers or museum staff. In some cases, the specimen, body parts and/or tissue samples were lodged with the local museum. Sub-samples of *Mola* spp. and *Ma. lanceolatus* tissue already held in museum collections in Australia and New Zealand were also provided. Collectively, all material registered at a museum, prior to or during our study, is herein referred to as 'museum specimens', with material obtained from other sources and not lodged with a museum is referred to as 'other material'. A summary of the longline biopsy samples is provided in Appendix A3.2. Details of museum specimens and other material are provided Appendix A3.1.

3.3.3 Molecular and phylogenetic analysis

Identifying Molidae specimens from the cytochrome *c* oxidase 1 (CO1) warrants caution, as only a small number of sequences (some mislabelled) are currently lodged with the Barcode of Life Data System (BOLD) (*https://www.boldsystems.org*) and Genbank (*https://www.ncbi.nlm.nih.gov/genbank/*). Furthermore, the potential basin-wide *M. mola* clades have not yet been identified in the CO1 locus (Nyegaard et al. 2018b [Chapter 2]), presently rendering the D-loop preferable for determining species identities.

Qiagen DNeasy Blood and Tissue Kits were used for DNA extraction, following the manufacturers protocol. The mtDNA D-loop was amplified using MolaA and MolaB primers (Yoshita et al. 2009). TaKaRa ExTaq Hot Start enzyme were used for the Polymerase Chain Reaction (PCR) with each reaction consisting of 0.13 µL TaKaRa enzyme, 2.5 µL 10xExTaq Buffer, 2 uL dNTP (2.5 mM each), 2 µL of each primers (10 pmol/uL), 16.4 µL water and 2 µL template (10 ng/µL) in each 25 µL reaction. PCR was performed using 36 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds. The PCR products were purified and Sanger sequenced by Macrogen. Sequences were checked and assembled in MacVector V.14.5.2.

The Molidae D-loop sequences from this study were pooled with sequences from Yoshita et al. (2009), Yamanoue et al. (2010), Yamanoue and Sawai (2012), Ahuir-Baraja et al. (2017) and Sawai et al. (2017). Some of our sequences were first reported in Nyegaard et al. (2018b) [Chapter 2] for the description of *M. tecta*. Sequence alignment, and assessment of optimal evolutionary model followed Nyegaard et al. (2018b); the HKY model (Hasegawa et al. 1985) with gamma distributed rate variation across sites, and a proportion of invariable sites (+I+G) scored lowest and second lowest according to the AICc and BIC, respectively. Phylogenetic relationships were inferred using Maximum Likelihood (ML), Bayesian and Maximum Parsimony (MP) analyses, following Nyegaard et al. (2018b), with *R. laevis* as outgroup and with 20,000,000 generations for the Bayesian analysis. The resulting ML phylogenetic tree was subjected to Poisson Tree Processes (PTP) modelling through the webserver (*http://species.h-its.org*) for inference of putative species boundaries (Zhang et al. 2013). The analysis was run with 500,000 generations and a burn-in of 0.3, and convergence was ascertained by visually inspecting the likelihood trace plot.

3.3.4 Review of museum specimens

The Molidae collections at the natural history museums in Australia and New Zealand were reviewed to verify the species identity of whole specimens and other identifiable material, except *R. laevis*, which was outside the scope of this study. The Melbourne, Perth, Auckland, Otago and Wellington museum collections were examined in person, while material from the Adelaide, Darwin, Hobart, Sydney, Brisbane, Christchurch, and Whanganui museums was examined via photographs and descriptions from examinations by museum staff or volunteers. The holotype of *M. ramsayi* (now *M. alexandrini*) at the London Natural History Museum (BMNH 1883.11.29.22), caught off New South Wales in 1882 (Giglioli 1883; Whitley 1931) and recently reviewed by Sawai et al. (2018), was also included in our study.

The morphology of museum specimens was assessed against Fraser-Brunner (1951), Nyegaard et al. (2018b) [Chapter 2] and Sawai et al. (2018) and identified to the lowest possible taxon, noting that the two *M. mola* clades cannot be distinguished morphologically from the currently available literature (Sawai et al. 2018). For specimens examined from photographs, as many characters as possible were assessed, and a specimen identification assigned when a subjectively satisfactory combination of clear traits was available. Some of these specimens were also included in Nyegaard et al. (2018b) [Chapter 2] and Sawai et al. (2018) for the description of *M. tecta*, and re-description of *M. alexandrini*, respectively, or included in the phylogenetic analysis by Yoshita et al. (2009).

3.3.5 Sampling latitude and sea surface temperature

The latitude of all specimens identified to species level, were pooled according to species identity. Sea surface temperature (SST) at the time of longline biopsy sampling, and sampling of other live specimens during 2013 – 14 and 2016 (i.e. excluding strandings and older records), were extracted through the Env-DATA Track Annotation Service on Movebank (*https://www.movebank.org*) (National Oceanographic and Atmospheric [NOAA] Modis Aqua OceanColor 4 km: 8 day averages using 'inverse-distance-weighted' interpolation). The sampling latitude and SST for each species, as well as SST and TL for the 'subtropical' and 'temperate' *M. alexandrini* groups, were tested for goodness of fit to a normal distribution with Shapiro-Wilks W tests. As the following data sets were not normally distributed: latitudes for *Ma. lanceolatus* ($W = 0.912$, $p < 0.01$) and *M. alexandrini* ($W = 0.955$, $p = 0.02$), SSTs for *M. alexandrini* ($W = 0.951$, $p = 0.05$), and SSTs and TLs for *M. alexandrini* 'temperate' group ($W = 0.922$, $p=0.02$; W0.913 $p=0.01$), the differences in median sampling latitude, SST and TL between groups were tested with non-parametric Mann-Whitney U tests. A summary of the analyses, with sample sizes and sources, is provided in Table 3.1. The mean annual SST data from 2013 – 14 were downloaded from NOAA (https://oceancolor.gsfc.nasa.gov), and the mean for 2013 and 2014 in each 4 km square calculated in Seadas 7.4.

Table 3.1 Source and number of specimens included in determination of species identity based on phylogenetics (phyl) and/or morpholy (morph), and in comparison of sea surface temperature (SST), latitude (Lat) or total length (TL) between species, as well as between 'subtropical' and 'temperate' groups within *Mola alexandrini*.

	Number of samples in each analysis						
	All species				***Mola alexandrini* groups**		
Source of samples/specimens	**Phyl**	**Morph**	**SST**[a]	**Lat**[a]	**Phyl**	**SST**	**TL**
Longline biopsy samples (*n* = 106)	106	0	105	105	47	47	46
Museum specimens (*n* = 45)	15	45	1	40	4	0	0
Other material (*n* = 12)	12	9	4	11	2	1	0
In total (all sources, *n* = 163)	133	54	110	156	53	48	46

[a]Excludes data from *Mola mola* specimens

3.3.6 Longline bycatch data

Molidae fisheries observer data from the ETBF (September 2001 – December 2013) and the New Zealand longline fishery (1987 – 2015) were provided by the AFMA and MPI, respectively. Both datasets consisted of 1) individual sunfish interactions (date, time, position, species); 2) observed fishing effort, which in Australia was total quarterly hook and line numbers in 1° latitude/longitude squares, and in New Zealand individual lines with date, time, position and number of hooks; and 3) quarterly commercial effort as total number of hooks and vessels in 1° latitude/longitude squares. Nearly 6 million hooks were observed during 2001 – 13 in Australia and ~11.4 million in New Zealand. All spatial coordinates were checked, and any data points without coordinates, on land or outside the fishing zones were cross-checked with the data providers, enabling all ambiguous sunfish interactions to be corrected. In the Australian data set, 13 records of observer effort (totalling 17,305 hooks = 0.3% of total observed) could not be resolved and were removed from further analysis.

The two data sets were merged and truncated to cover the same time period, September 2001 – December 2013, and calculations made of 1) the annual average CPUE, as number of sunfish.1,000 observed hooks^{-1} in 1° latitude/longitude grids across all years, and 2) the average quarterly observed CPUE (2001-13) during January – March (Q1), April – June (Q2), July – September (Q3), and October – December (Q4). The CPUE calculations from any 1° square in which less than five (Australia) or three (New Zealand) boats had fished during each quarter, with or without observer coverage, were removed from the analysis to ensure industry confidentiality. This lead to the omission of 49 squares in Australia (mostly in Q1 and Q4) and 54 squares in New Zealand (mostly in Q1 to Q3).

Four subsets of observer data were selected to represent subtropical, warm-temperate and cold-temperate regions of the ETBF and New Zealand fishing grounds, respectively. These areas were chosen on the basis of high relative fishing pressure (Figure 3.1), likely being dominated by a single sunfish species (see Section 3), as well as having reasonably high observer cover (> 10,000 annual observed hooks) during most years (maximum two exceptions) in a small number of adjacent 1° squares. The mean annual CPUE was calculated within each of these areas and the trend in sunfish CPUE investigated over time with Mann-Kendall Tau tests (T) (Mann 1945; Kendall and Gibbons 1990), which may be viewed as a nonparametric test for a slope of linear regression differing from zero for time-ordered data (Gilbert 1987).

3.4 Results

3.4.1 Phylogenetic specimen identification

A total of 106 tissue samples were recovered from the three longline fisheries: 30 from New Zealand, 63 from the ETBF, and 13 from the WTBF (Table 3.2). In addition, 21 samples from museum collections and other sources across Australia and New Zealand were obtained (Appendix A3.1). Except for a small number of these samples (*n* =19), which were first reported in Nyegaard et al. (2018b) [Chapter 2], the D-loop sequences were uploaded to GenBank under Accession numbers MG253927 – MG254034.

The trimmed alignment of the Molidae sequences comprised 843 characters, with 260 parsimony informative sites. The phylogenetic trees inferred by ML, MP and Bayesian methods displayed similar topographies, with no differences in the relationships of the major lineages, and as expected, resembled those of other studies (e.g. Yoshita et al. 2009; Ahuir-Baraja et al. 2017; Nyegaard et al. 2018b [Chapter 2]). Each of the clades, *Ma. lanceolatus*, *M. alexandrini*, *M. tecta*, *M. mola* (Pacific) and *M. mola* (Atlantic), had high branch supports in all phylogenetic analyses (98 – 100 ML, 0.98 – 1.0 PP and 70 – 100 MP), and were also supported at the putative species level by PTP analysis (Figure 3.2).

Most of our tissue samples nested in the *Ma. lanceolatus* and *M. alexandrini* clades, with a smaller number in the *M. tecta* clade (Figure 3.2). In addition, one sample nested in the *M. mola* Atlantic clade, and two in the *M. mola* Pacific clade. Within the *M. alexandrini* species clade, 16 samples nested in the 'subtropical' group of Yoshita et al. (2009). Similar to Yoshita et al. (2009) and Ahuir-Baraja et al. (2017), this clade had high branch support (80 ML, 1 PP and 86 MP), but was not supported at the putative species level by PTP analysis.

Table 3.2 Number of biopsy samples and whole specimens identified to species level, from longline bycatch, museum collections and other sources in New Zealand and Australia, including the original species identity assigned at the time of sampling or by the museum. ETBF and WTFB are Eastern and Western Tuna and Billfish Fishery, respectively. Further specimen details are given in Appendix A3.1.

Source	**Species ID (this study)**	**Breakdown of original species ID, assigned by the sampler or museum**
Longline biopsies, New Zealand (*n* = 30)	22 *Mola alexandrini*	22 as *Mola mola*
	8 *Mola tecta*	8 as *M. mola*
Longline biopsies, Australia (ETBF) (*n* = 63)	14 *M. alexandrini*	12 as *M. mola*
		2 as Unspecified Molidae
	49 *Masturus lanceolatus*	29 as *Ma. lanceolatus*
		8 as *M. mola*
		12 as Unspecified Molidae
Longline biopsies, Australia (WTBF)	11 *M. alexandrini*	10 as *Mola* sp.
(*n* = 13)		1 as *Ma. lanceolatus*
	1 *M. mola* (Atlantic clade)	1 as *Mola* sp.
	1 *Ma. lanceolatus*	1 as *Ma. lanceolatus*
Museum specimens, Australia and New	19 *M. alexandrini*	9 as *M. ramsayi*[a]
Zealand (*n* = 45)		9 as *M. mola*
		1 as *Mola* sp.
	2 *M. mola*	1 as *M. ramsayi*[a]
		1 as *Mola* sp.
	19 *M. tecta*	4 as *M. mola*
		11 as *M. ramsayi*[a]
		3 as *Mola* sp.
		1 as *Mola* sp. nov.
	5 *Ma. lanceolatus*	1 as *M. ramsayi*[a]
		4 as *Ma. lanceolatus*
Other sources, Australia and New Zealand	2 *M. alexandrini*	1 as *Mola* sp.
(n = 12)		1 as Molidae
	1 *M. mola*	1 as *M. mola*
	9 *M. tecta*	9 as *Mola* sp. novo

[a] Now *Mola alexandrini* (Sawai et al. 2018)

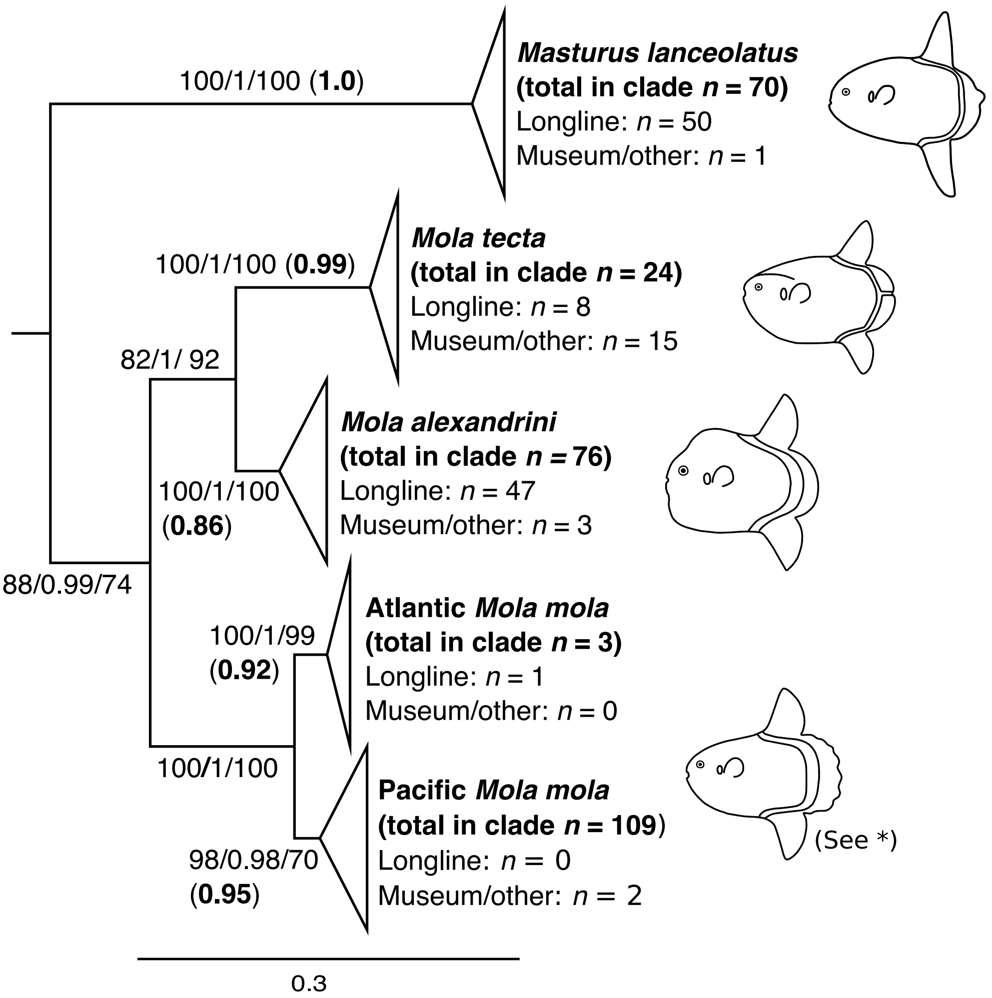


Figure 3.2 Phylogenetic relationships inferred by Maximum Likelihood (ML) of Molidae mitochondrial D-loop sequences from Yoshita et al. (2009), Yamanoue et al. (2010), Yamanoue and Sawai (2012), Ahuir-Baraja et al. (2017), Nyegaard et al. (2018b) [Chapter 2], Sawai et al. (2017) and this study (from longline bycatch and museum/other sources). Clades supported at the putative species level by Poison Tree Processes (PTP) are shown as collapsed sequences. Numbers above or below branches refer to ML bootstrap values (left), Bayesian posterior probabilities (middle) and Maximum Parsimony bootstrap values (right), with PTP putative species support (ML partition) in bold in parentheses. Branch length for outgroup *Ranzania laevis* not shown. Scale bar represents nucleotide substitution per site. Fish illustrations based on Nyegaard et al. (2018b) [Chapter 2], Sawai et al. (2018) and Sawai (unpublished). *Morphology represents large Pacific *Mola mola*.

Across the WTBF, ETBF and New Zealand, 47% of the biopsy sampled sunfish were identified at the time of sampling as *M. mola*, 29% as *Ma. lanceolatus*, ~10% as *Mola* sp. and 14% as Unspecified Molidae (Table 3.2). Genetic analyses, however, found that most individuals were *Ma. lanceolatus* (47%) and *M. alexandrini* (44%), a small number were *M. tecta* (8%) and very few were *M. mola* (~1% [1 of 106 sunfish]). A total of 60% of *Ma. lanceolatus* were identified correctly at sea, however other *Ma. lanceolatus* were identified as *M. mola* or Unspecified Molidae.

3.4.2 Morphological specimen identification

The museum collections included specimens of a wide range of sizes (29.3 – 254 cm TL, excluding larvae and small juvenile specimens, which were outside the scope of this study). Most of these specimens had been obtained by the museums over a number of years and seasons, with a small subset registered during our study period (*n* = 6). Of these, only one had been collected specifically for its identity (*M. tecta* holotype NMNZ P.057679 in Nyegaard et al. 2018b [Chapter 2]). In total, 47 museum specimens were reviewed, and 45 species identities were resolved (Table 3.2). Two specimen (CMC F228 and TMAG D3885) could not be resolved from the photos provided; seven specimens were not examined due to access issues (e.g. Table A3.1.1 in Appendix A3.1).

Overall, 19 *M. tecta*, 19 *M. alexandrini*, five *Ma. lanceolatus* and two *M. mola* were found in the museum collections. Several of these were included in Nyegaard et al. (2018b) [Chapter 2] or Sawai et al. (2018) for the description of *M. tecta* and re-description of *M. alexandrini*. In total, species identities were re-assigned to 26 specimens, and resolved for six specimens, which had originally been identified to genus level only (Table 3.2; Table A3.1.1 in Appendix A3.1). Specimens which were identified as *M. alexandrini* in this study had in many cases been identified correctly as *M. ramsayi* (47%), while others had been identified as *M. mola* (47%) or *Mola* sp. (5%). Four of five *Ma. lanceolatus* had been identified correctly, while all *M. tecta* specimens had been identified as *M. ramsayi*, *M. mola* or *Mola* sp., prior to its description in 2017 (Nyegaard et al. 2018b [Chapter 2]).

Of the 12 specimens from the other material, seven were from strandings, one was handed in by a commercial purse seine vessel, and one was sampled from a piece of severed clavus retained after an accidental propeller strike. An additional two whole *M. tecta* specimens were collected and euthanized by a fisheries observer in the New Zealand longline fishery, specifically collected for their species identities (NZ18, NZ19 in Nyegaard et al. 2018b [Chapter 2]). Other material also included one D-loop sequence from a New Zealand specimen first reported by Yamanoue and Sawai (2012). Overall, the material consisted of 9 *M. tecta*, 2 *M. alexandrini* and 1 Pacific *M. mola* (Table 3.2; Table A3.1.2 in Appendix A3.1).

3.4.3 Spatial distribution

The majority of longline samples from the ETBF were recovered off Queensland (59 of 63 samples), and consisted predominantly of *Ma. lanceolatus* (*n* = 48), with a smaller number of *M. alexandrini* (*n* = 11); four ETBF samples were recovered off New South Wales; three *M. alexandrini*, and one *Ma. lanceolatus* (Table 3.2, Figure 3.3A,B). In the WTBF, all samples were recovered off southwestern Australia, and consisted mainly of *M. alexandrini* (*n* = 11), with one *Ma. lanceolatus* and one Atlantic *M. mola* (Table 3.2, Figure 3.3A,B,C). All 30 samples from New Zealand were recovered off the northeast coast of the North Island, and consisted mainly of *M. alexandrini* (*n* = 22), with a smaller number of *M. tecta* (*n* = 8) (Figure 3.3B,C).

The five *Ma. lanceolatus* specimens found in the museum collections had been obtained between 1981 and 2017, mainly from the southern part of our study area; two had stranded on the southeast coast of Australia (Victoria and South Australia) and one in northern New Zealand, with a fourth taken by a purse seiner north of New Zealand (Figure 3.3A) (Paulin et al. 1982). A single specimen had stranded in the tropics (Northern Territory).

The *M. alexandrini* museum specimens and other material, obtained between 1882 and 2015, showed a similar distribution to the *M. alexandrini* longline samples (Figure 3.3B) with records covering a large geographical region. A small stranded specimen in southern New Zealand represented the southernmost extent of this species in our study, with several longline samples off Queensland representing the northernmost encounters.

Mola tecta were relatively common among museum and other material (*n* =28), obtained between 1952 and 2017. The material was partly collected from the north coast of New Zealand, overlapping with the area where *M. tecta* was found among the longline samples (Figure 3.3C). However, the majority of the *M. tecta* material (~60%), originated from the South Island of New Zealand and south-eastern Australia, outside the areas of longline sampling.

The three *M. mola* identified in museum collections and other material consisted of a cast from a large specimen (254 cm TL) stranded on the South Island of New Zealand in 1930; a large specimen (~200 cm TL) stranded in Victoria in 2003, and a large specimen (261 cm TL) stranded on the South Island of New Zealand in 2011. The tissue samples available from the latter two specimens nested in the *M. mola* Pacific clade (Figure 3.3D).

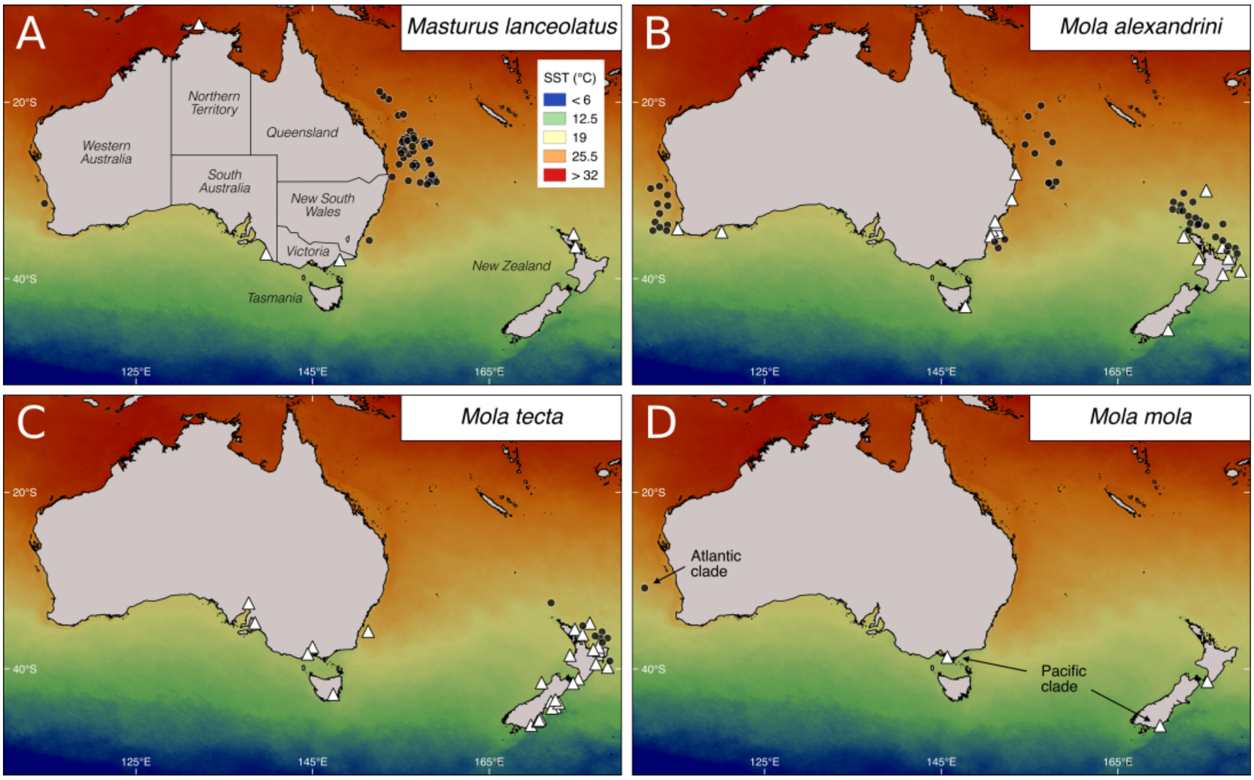


Figure 3.3 Locations of four Molidae species **A)** *Masturus lanceolatus*; **B)** *Mola alexandrini*; **C)** *Mola tecta*; and **D)** *Mola mola*, from longline bycatch in Australia and New Zealand (black circles), and museum collections and other material (white triangles), identified to species level phylogenetically and/or morphologically. Note some locations have been altered slightly to avoid complete overlap with others. Sea surface temperatures (SST) are average Modis-Aqua SST in 4 km squares during 2013 – 14.

3.4.4 Temporal distribution and sizes of sunfish

In the ETBF, samples of both *Ma. lanceolatus* and *M. alexandrini* were obtained throughout the sampling period, with the majority recovered during September 2013 – February 2014 (Figure 3.4). The approximate TL of sampled *Ma. lanceolatus* ranged between 80 – 210 cm (mean ± 1 SE = 128 ± 4 cm; *n* = 49), and *M. alexandrini* between 100 – 220 cm (138 ± 11 cm; *n* = 14). Sampling in the WTBF took place during two months in 2014 (Figure 3.4); the approximated TL of *M. alexandrini* ranged between 50 – 200 cm (125 ± 14 cm; *n* = 10), and the single Atlantic *M. mola* and *Ma. lanceolatus* specimens were an estimated 120 and 150 cm TL, respectively. In New Zealand, observer sampling took place mainly during the peak longline fishing season in 2013 (Figure 3.4). The TL of *M. alexandrini* in New Zealand ranged between 81 – 175 cm (125 ± 6 cm, *n* = 22) and between 50 – 150 cm (95 ± 14 cm, *n* = 8) for *M. tecta*.

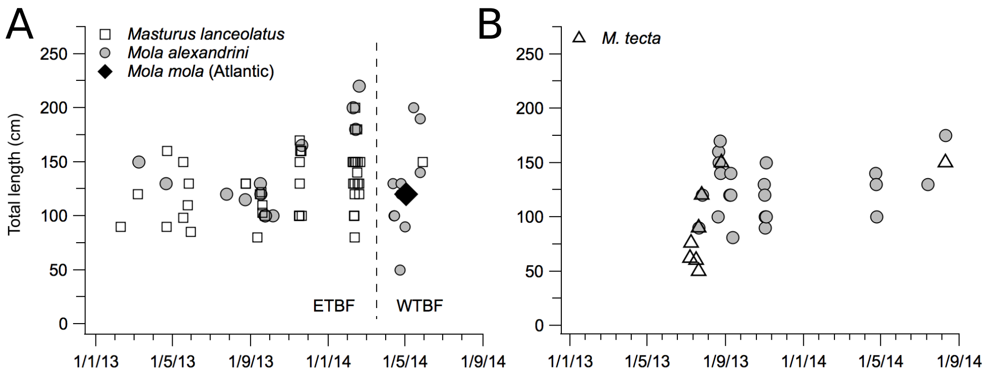


Figure 3.4 Date and estimated total length (cm) of each sunfish sampled on commercial longline vessels in the **A)** Eastern and Western Tuna and Billfish Fishery (ETBF and WTBF, respectively), and **B)** New Zealand longline fishery. Open boxes: *Masturus lanceolatus*; grey circles: *Mola alexandrini*; black circle: *Mola mola* (Atlantic clade); open triangles: *Mola tecta*.

3.4.5 Sampling latitude and sea surface temperature

The range of latitudes associated with *Ma. lanceolatus* (~11 – 38°S) and *M. alexandrini* (~20 – 46°S) from all sources were wider than for *M. tecta* (~33 – 46°S), with overlaps in ranges between all species (Figure 3.5A). The median latitude of *Ma. lanceolatus* (~26°S) was significantly lower (i.e. closer to the equator) than that of *M. alexandrini* (~34°S; Mann-Whitney 2-tail: $U_{56, 65} = 547$; $p < 0.01$), which was in turn significantly lower than that of *M. tecta* (~38°S; $U_{65, 37} = 295$ $p < 0.01$).

The SST during sampling of live individuals (2013 – 14, 2016) (i.e. excluding strandings and older material) differed markedly among *Ma. lanceolatus*, *M. alexandrini* and *M. tecta*. The SST ranged between 21.5 – 27.2°C (mean ± 1 SE = 24.7 ± 0.2°C, *n* = 50) for *Ma. lanceolatus*, 16.2 – 26.9°C (20.4 ± 0.4°C, *n* = 48) for *M. alexandrini* and 15.0 – 17.9°C (16.7 ± 0.2°C, *n* = 12) for *M. tecta* (Figure 3.5B). The SST for the single Atlantic *M. mola* sampled off Western Australia was 21.0°C. The SST range for *M. alexandrini* was much wider than for the other two species, overlapping with each of them at either end of the range. The median SST during sampling of *Ma. lanceolatus* (24.9°C) was significantly warmer than for *M. alexandrini* (20.4°C; Mann-Whitney 2-tail: $U_{50, 48} = 242$; $p < 0.01$); and that of *M. alexandrini* was in turn significantly warmer than that of *M. tecta* (16.8°C; $U_{48, 12} = 52$; $p < 0.01$). A significant difference was also found between the median SST during sampling of the 'subtropical' (21.5°C) versus 'temperate' (18.7°C) *M. alexandrini* groups ($U_{15, 33} = 363$; $p < 0.01$) (Figure 3.6A). No significant difference was found between the estimated TL of the two groups (median of 'subtropical' group = 130 cm TL; 'temperate' group = 120 cm TL; $U_{13, 33} = 233$; $p = 0.33$) (Figure 3.6b). Geographically, the two *M. alexandrini* groups were found sympatrically in the

Indian Ocean, as well as in the Pacific Ocean, however the ‘subtropical’ group was most common among the Indian Ocean samples (75% of all *M. alexandrini* genetic samples [*n* = 12]), and the ‘temperate’ group was most common among the Pacific Ocean samples (83% of all genetic samples [*n* = 40]) (Figure 3.6B).

3.4.6 Catch per unit effort (CPUE)

The annual mean observer sunfish CPUEs (2001 – 13) in 1° latitude/longitude squares across the ETBF and New Zealand longline fishing grounds (Figure 3.7A) consisted predominantly of zero interactions (31 and 28% of all squares in Australia and New Zealand, respectively), and CPUEs of < 1 sunfish.1,000 hooks^{-1} (63 and 41% of all squares, respectively). In Australia, few squares had mean annual CPUEs of > 2 sunfish.1,000 hooks^{-1} (2% of squares), whereas this was more common in New Zealand (18% of squares), in particular along the northeast coast of New Zealand (between 32 – 38°S), where CPUEs of 3 – 6.5 sunfish.1000 hooks^{-1} were common. Similarly high sunfish bycatch rates (3 – 4.5 sunfish.1000 hooks^{-1}) were also found, albeit to a lesser degree, off the border between New South Wales and Victoria (37°30’ S 149°58’E) (Figure 3.7A). Generally, no or very few interactions were recorded offshore from the Great Barrier Reef Marine Park in Queensland (above 20°S).

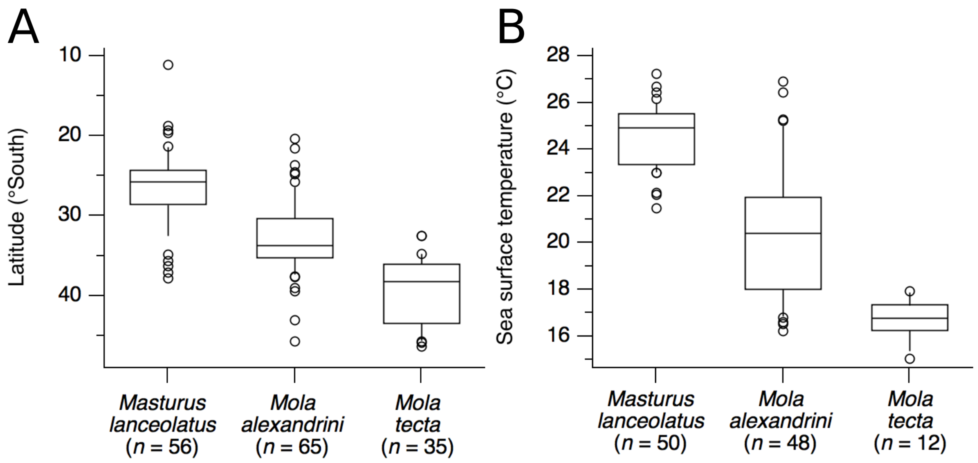


Figure 3.5 **A)** Latitude of longline samples, museum specimens and other material obtained between 1882 –2017, and **B)** MODIS Sea Surface Temperature (SST) during sampling of longline and other live specimens during 2013 – 14, 2016 (i.e. excluding strandings and older material) of the three large ocean sunfish species *Masturus lanceolatus*, *Mola alexandrini* and *Mola tecta*. Middle of box: median; upper and lower box edges: 75 and 25 percentiles, respectively; top and bottom whiskers: 90 and 10 percentiles, respectively; open circles: data points beyond 90 and 10 percentiles, respectively.

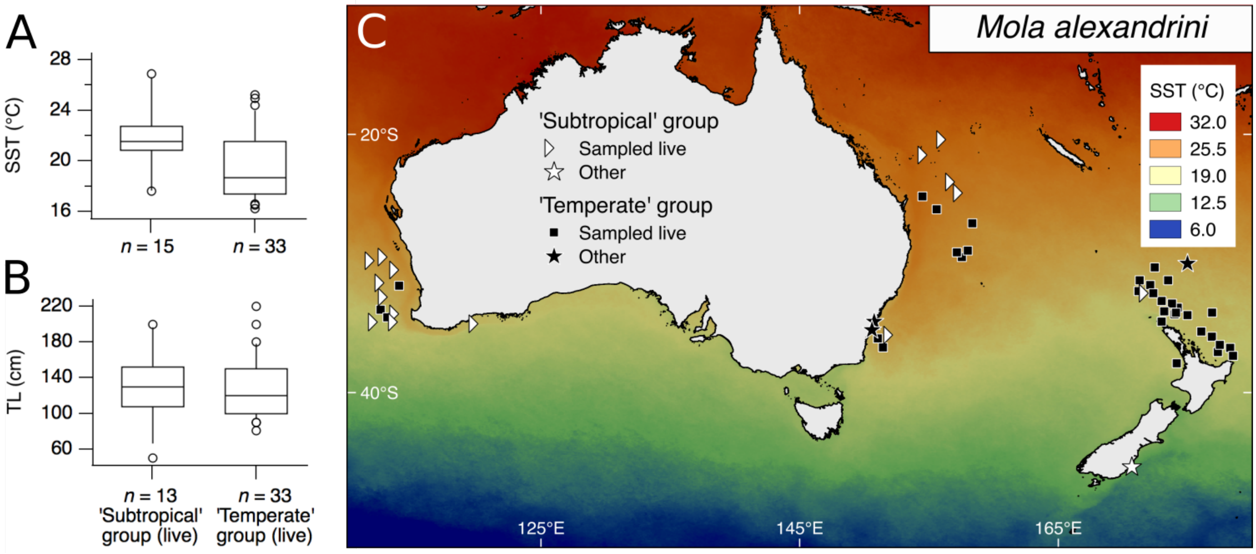


Figure 3.6 Box plots of **A)** satellite sea surface temperature (SST) at the time of sampling, and **B)** estimated total length (TL) of *M. alexandrini* specimens sampled in the longline fisheries in Australia and New Zealand, or otherwise sampled live (2013 – 14, 2016), nesting in either the 'subtropical' or 'temperate' phylogenetic mtDNA D-loop groups of Yoshita et al. (2009). Middle of box: median; upper and lower box edges: 75 and 25 percentiles, respectively; top and bottom whiskers: 90 and 10 percentiles, respectively; open circles: data points beyond 90 and 10 percentiles, respectively; **C)** *Mola alexandrini* 'subtropical' (white) and 'temperate' (black) groups superimposed on average SST in 4 km squares during 2013 – 14. Stars represent older samples, not included in the SST or TL analysis in A) and B).

The spatial distributions of quarterly mean CPUEs (2001 – 13) across the ETBF and New Zealand fishing grounds were similar among the four quarters (Figure 3.7C). The highest CPUEs were consistently found off the northeast cost of New Zealand. Similarly high CPUEs off southeast Australia (Figure 3.7A) were recorded during the April – June quarter (Figure 3.7B – D), with no interaction data available from this area for other quarters. The data on sunfish interactions from the South Island of New Zealand were mainly from the seasonal fishery during the April – June quarter.

The annul mean CPUEs in Areas 1 and 2 off Queensland and New South Wales (Figure 3.7F; Figure 3.8B,C), respectively, were similar in magnitude, while the CPUEs in Areas 3 and 4 off the northeast and southwest of New Zealand (Figure 3.7F; Figure 3.8D,E) were markedly higher and lower, respectively, than those off Queensland and New South Wales. All CPUE time series showed large fluctuations between years with no obvious pattern of decline or increase over time, which was confirmed by the lack of significance for the relationship between catch rates and year from the Mann-Kendall T tests (Area 1 – T_{12} = 0.273, p = 0.22; Area 2 – T_{12} = -0.273, p = 0.22; Area 3 – T_{15} = 0.086, p = 0.66; Area 4 – T_{16} = 0.20, p = 0.28).

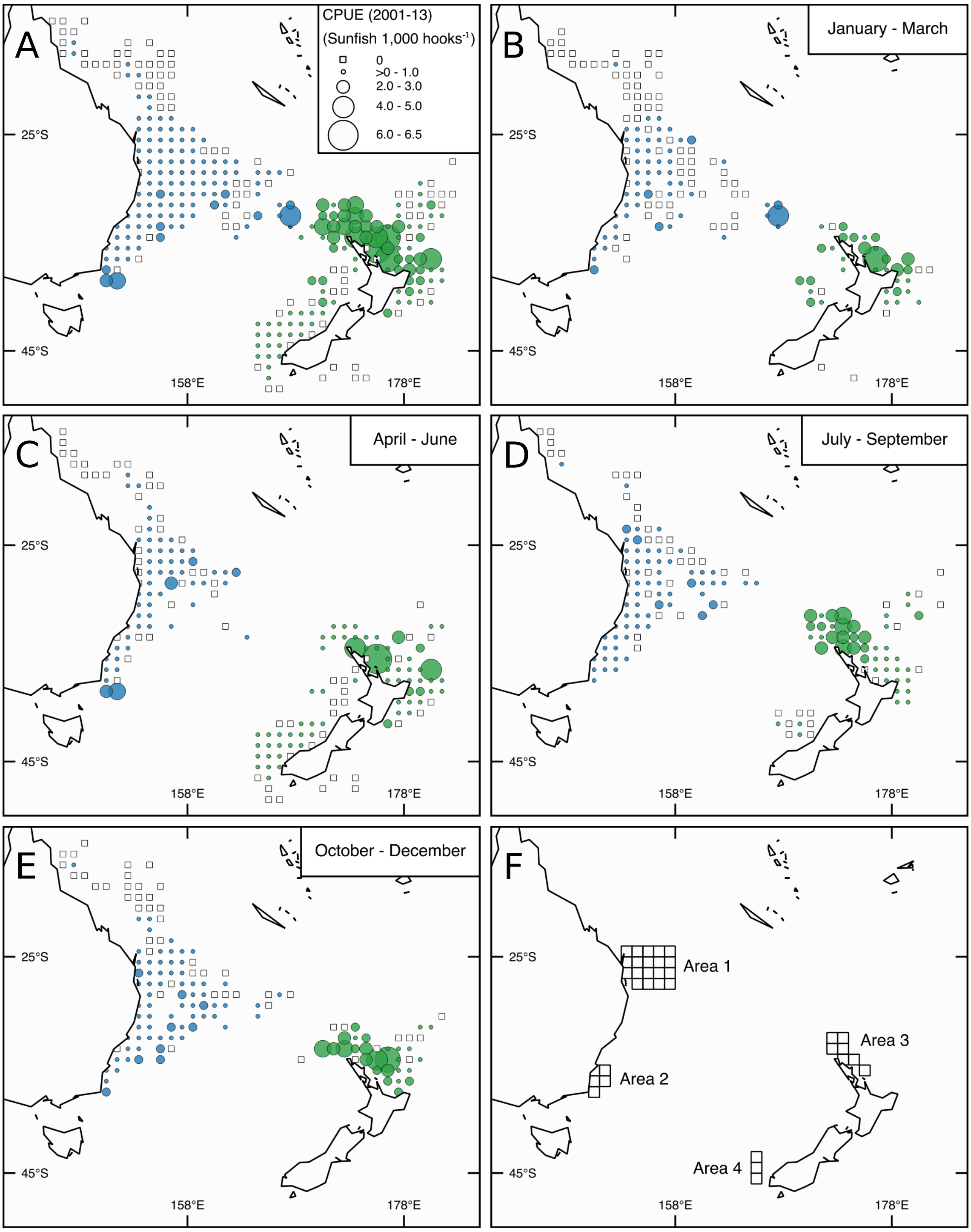


Figure 3.7 Average catch per unit effort (2001 – 13) (CPUE; sunfish.1,000 observed hooks^{-1}) in the Eastern Tuna and Billfish Fishery in Australia (blue), and longline fishery in New Zealand (green) in 1° latitude/longitude squares with fishing activity of commercial vessels ≥ 5 (Australia) or ≥ 3 (New Zealand). **A)** annual average; **B)** January-March; **C)** April-June; **D)** July-September; and **E)** October-December; **F)** Areas 1 – 4 comprised of adjacent 1° squares.

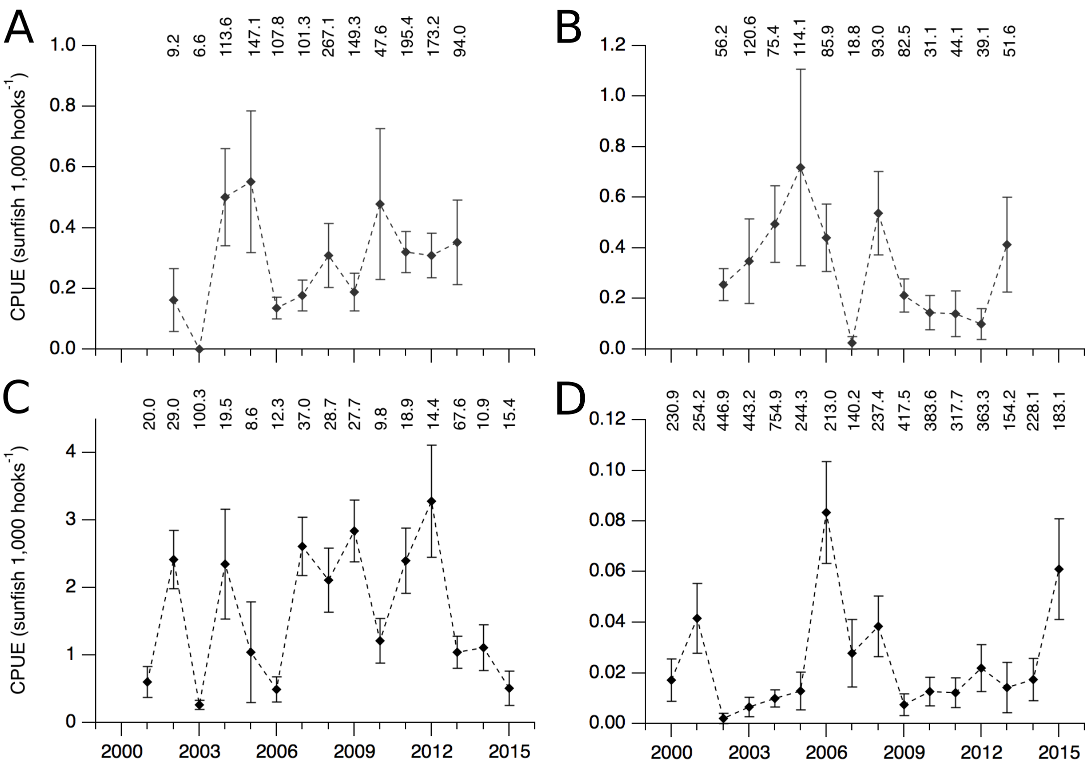


Figure 3.8 Average annual sunfish Catch per Unit Effort (CPUE) over time from fisheries observer data collected off the coast of **A)** Queensland (Area 1); **B)** New South Wales (Area 2); **C)** north New Zealand (Area 3); and **D)** southwest New Zealand (Area 4) in the Australian and New Zealand longline fisheries. Errors bars represent 1 SE of average CPUE in adjacent 1° latitude/longitude squares (Australia: Area 1 and Area 2), or of average fishing line CPUE within the overall area (New Zealand: Area 3 and 4). Total observed hooks (in thousands) as 90° rotated numbers.

3.5 Discussion

Genetic analysis of sunfish tissue samples from the Australian and New Zealand longline fisheries revealed a mix of species, comprising mostly *Ma. lanceolatus* and *M. alexandrini* with a small number of *M. tecta*, and a single *M. mola* (Atlantic clade). The museum specimens and other material corroborated the longline results, and added valuable information about species distribution from areas with few or no longline samples. The results revealed that specimens were commonly mis-identified by fisheries observers and in museum collections. Overall, the stranding/sampling latitude of sunfish from all sources differed markedly between species, with *Ma. lanceolatus* overall found at significantly lower latitudes than *M. alexandrini*, which was again found at significantly lower latitudes than *M. tecta*. The SSTs during sampling of live specimens also differed significantly among the three most common species, with *Ma. lanceolatus* associated with warmer surface waters than *M. alexandrini*, which was in turn associated with warmer surface waters than *M. tecta*. Geographically, *Ma. lanceolatus* was the most common species in the tropical/subtropical waters off Queensland, while *M. alexandrini* dominated in the warm-temperate areas off New South Wales, the north coast of New Zealand, and off southwest Western Australia. *Mola tecta* was among the longline samples from the North Island of New Zealand, with museum specimens and other material confirming its presence here, and furthermore suggesting that it is the most common species in the cold-temperate areas of New Zealand and southeastern Australia. *Mola mola* was infrequent among longline samples, museum specimens and other material, however both genetic clades were found, namely one Atlantic *M. mola* in the Indian Ocean, and two Pacific *M. mola* in the Pacific Ocean.

Both the 'subtropical' and 'temperate' *M. alexandrini* groups were found in Australia and New Zealand, with a dominance of the former among the Indian Ocean samples, and the latter among the Pacific Ocean samples. Slightly higher SSTs were associated with sampling of the 'subtropical' group compared to the 'temperate' group. These groups were not supported on the putative species level.

The observed annual mean CPUEs (2001 – 13) of sunfish were relatively low and uniform across the Eastern Tuna and Billfish Fishery (ETBF) in Australia, with the exception of a small area off southeastern Australia with slightly greater CPUEs. In New Zealand, the observed annual mean CPUEs were much greater off the northeast coast of the North Island, compared with Australia and the more southern waters of New Zealand. No large-scale spatial shifts in quarterly mean CPUEs were evident in either fishery. The annual mean CPUE time series between 2001 and 2013 (Australia), and between 2000 – 2015 (New Zealand), did not show any discernible trend over time within the four fishing ground subareas; i.e. in subtropical Australia (Area 1), warm-temperate Australia and New Zealand (Area 2 and 3, respectively), and cold-temperate New Zealand (Area 4).

3.5.1 Distribution – latitude and sea surface temperature

Within our study area, the three most common sunfish species, *Ma. lancaolatus*, *M. alexandrini* and *M. tecta*, were found at significantly different latitudes and sea surface temperature regimes. The relatively low latitudes and high SSTs associated with *Ma. lanceolatus* correspond well with this being a tropical species (Liu et al. 2009; Froese and Pauly 2018). *Mola alexandrini* and *M. tecta*, in turn, appeared to be subtropical/warm-temperate and cold-temperature species, respectively, corresponding well with recent global sunfish habitat modelling, which suggested that the *Mola* species complex avoid high SSTs in the tropics during summer months (Phillips et al. 2017).

Sea surface temperature separation between sympatric *Mola* species, as found for *M. alexandrini* and *M. tecta* in this study, was also reported for *M. alexandrini* (mean = 19.9°C, range = 16.8 – 25.6°C, *n* = 12) and Pacific *M. mola* (17.7°C, 11.5 – 25.6°C, *n* = 157) in the Sanriku region, Japan (Sawai et al. 2011). The Japanese *M. alexandrini* SST findings were remarkably similar to ours, confirming a wide SST range for this species in both the northwest and southwest Pacific. Moreover, our results are also consistent with Yoshita et al. (2009), whereby the 'subtropical' *M. alexandrini* clade was associated with significantly higher SST than the 'temperate' group. Further sampling is needed to verify if these groups have different thermal optima, or if the differences are an artefact of skewed geographical and temporal sampling of two different populations.

The SST ranges found for each sunfish species in this study should not be interpreted as 'global ranges', as our data came from a limited area compared with the wide geographical distributions of each species, and furthermore make no assumptions of the sub-surface ocean stratification and thermal regimes, and the sunfish utilisation and occupation of these waters. It is well established that sunfish have impressive thermal tolerances (Dewar et al. 2010), with tagging studies consistently showing that *M. mola*, *M. alexandrini* and *Ma. lanceolatus*, experience large temperature fluctuations as they repeatedly dive to several hundred meters, below the thermocline, to forage (e.g. Seitz et al. 2002; Dewar et al. 2010; Potter et al. 2011; Nakamura et al. 2015; Thys et al. 2016). Several studies have linked the distribution and migration of *Mola* spp. to certain SST regimes (e.g. Dewar et al. 2010; Sousa et al. 2016b; Phillips et al. 2017), and any such association with SST is probably linked to the periodic 'basking' at the sea surface in between foraging dives, critical to thermoregulation (Nakamura et al. 2015). Factors other than thermoregulation may also compel sunfish to occupy relatively shallow waters in between deep dives, such as recovering from hypoxia during feeding below the thermocline (Cartamil and Lowe 2004; Sims et al. 2009b; Thys et al. 2015). In addition, sunfish are known to seek out cleaner fish in relatively shallow waters (e.g. Konow et al. 2006; Thys et al. 2017) and seabirds at the sea surface (Abe and Sekiguchi 2012; Abe et al. 2012) to manage skin parasites, although the frequency of such activities are not known. Importantly, however, sunfish diving behaviour appears to be very flexible in response to environmental conditions and prey distributions (e.g. Hays et al. 2009; Sims et al. 2009b; Sousa et al. 2016b). The wide distribution of each sunfish species (Nyegaard et al., 2018b [Chapter 2]; Sawai et al., 2018), as well as the large geographic overlaps between them, probably result from an ability to balance thermal, oxygen and prey-seeking budgets in variable oceanic conditions through a range of behaviours including deep diving, sea surface basking and flexibility in utilising favourable conditions in sub-surface mixed layers for thermoregulation purposes (e.g. Cartamil and Lowe 2004; Potter e al. 2011). Further research is needed to understand the drivers of niche partitioning of the large sunfish species, which may plausibly include differing thermal optima and tolerances of low temperatures and hypoxic conditions, as for some sympatric tuna species (e.g. Mislan et al. 2017).

3.5.2 Distribution – ocean currents

The distributions of *Ma. lanceolatus*, *M. alexandrini* and *M. tecta* in our study area differed between species in both latitude and SST, however large geographical overlaps were also seen, plausibly linked to the major surface current systems in the region. These include a warm pole-wards flowing boundary current along both the east (East Australian Current, EAC) and west coast (Leeuwin Current) of Australia, and cold-water influences from the Southern Ocean along the southern coast lines of Australia and New Zealand. Briefly, the EAC brings subtropical water south along the east coast of the Australian continent, with an extension periodically reaching as far south as Tasmania; an eastward arm of the EAC around 30 – 35°S creates the Tasman Front, which extends towards and along the northeast and Southeast coast of New Zealand, where it is eventually forced eastwards by the Chatham Rise (Gordon et al. 2010a; Sutton and Bowen 2014; Chiswell et al. 2015). Combined, this flow path of subtropical water may explain the wide north-south range of *M. alexandrini* from the tropics of Queensland (i.e. above ~23°27'S) to the warm-temperate north and north-eastern New Zealand (~37°S), and its occasional occurrences further south (e.g. Tasmania, South Island of New Zealand). This current system may also explain the occasional presence of *Ma. lanceolatus* well south of the tropics, including the north coast of New Zealand (Figure 3.3A). Conversely, the southern coastline of Australia and southern New Zealand are influenced by sub-polar water masses from the Antarctic Circumpolar Current (Chiswell et al. 2015). While this system shows large seasonal and inter-annual variation in its strength and distribution (Graham and De Boer 2013), the generally colder surface waters in these areas correspond well with the occurrence of *M. tecta* off southern New South Wales, Tasmania, Victoria, South Australia and New Zealand.

Like the EAC, the Leeuwin Current brings subtropical water south along Western Australia's coastline. The strength of this current varies markedly between seasons and years, at times rounding Cape Leeuwin off southwestern Australia, extending along the Great Australian Bight towards (and sometimes reaching) the west coast of Tasmania (Feng 2003; Ridgway and Godfrey 2015). The limited number of samples (*n* = 14) obtained from the Indian Ocean during this study were all collected off southwestern Australia during a limited time period (~2 months), and were dominated by *M. alexandrini*. Notwithstanding these limitations, it seems plausible that this species may have a wide range from the north of Western Australia to Cape Leeuwin, and along the south coast of Western Australia, similar to the wide distribution off the east coast of Australia and New Zealand. *Mola alexandrini* has indeed been confirmed from tropical Western Australia (i.e. above ~23°27'S), as well as Indonesia (mainly from the cold water upwelling areas off Bali and Alor) (Thys et al. 2016; Sawai et al. 2018). Further research is needed to ascertain its presence along the Great Australian Bight (Figure 3.1), which may be sporadic, and depend on the strength of the Leeuwin Current. *Mola tecta* was not among the relatively few Indian Ocean samples, but we expect it may occur off southwestern Australia, at least sporadically in colder years. Only one *Ma. lanceolatus* was sampled off Western Australia, but this species is presumably common in the tropical waters off western and northern Australia, with a southwards range extension associated with the Leeuwin current, akin to the east coast of Australia.

In the Northern Hemisphere, the occurrence of sunfish has similarly been linked to the influence of ocean currents. Specifically, sunfish (reported as *M. mola*) off Iceland were associated with the North Atlantic Drift, an extension of the Atlantic Current transporting warm waters north into the Iceland Basin. Here, seasonal sunfish occurrences were found during the warmest months of the year, and inter-annual differences in sunfish numbers were attributed to the warmer years of a positive Atlantic Multidecadal Oscillation (Palsson and Astthorsson 2017). Likewise, sunfish (reported as *M. mola*) occurring along the coast of Norway likely represents a northern range extension through “intentional, incidental or accidental” northwards movements, associated with the warm waters of the North Atlantic Drift and Norwegian Coastal Current (Frafjord et al. 2017).

3.5.3 Potential bias in interpreting distribution patterns

Seasonal migratory movements of *M. mola* between low (winter) and high (summer) latitudes in the north Atlantic have been described in several studies (e.g. Sims et al. 2009b; Potter et al. 2011; Sousa et al. 2016b; Breen et al. 2017). It is possible that similar seasonal movements in our study area could have caused an over- or under-representation of one or more sunfish species in our longline samples, thereby potentially explaining the surprising paucity of *M. mola*. However, the overall congruence with museum collections and other material strongly suggests that *M. mola* is relatively uncommon off eastern Australia and New Zealand, and that this area is dominated by the three species *Ma. lanceolatus*, *M. alexandrini* and

M. tecta. The limited data from southwestern Australia likewise indicates that *M. mola* is relatively uncommon here. However further research is needed to verify this, as well as to investigate the vast coastlines and offshore areas of Australia and New Zealand not covered in this study.

3.5.4 Sunfish sizes

In this study, *Ma. lanceolatus*, *M. alexandrini* and *M. tecta* from all sources included both small (< 1 m TL) and large (> 2 m TL) specimens, while the three *M. mola* specimens from Pacific Australia and New Zealand were all large (> 2 m TL). This contrasts with the sunfish size distribution found in Japanese and North Pacific waters, where Pacific *M. mola* was common among the sampled sunfish (n = 97) and had a wide range of sizes, whereas *M. alexandrini* was less common (n = 17) and were all large (> 1.80 m TL) (Yoshita et al. 2009; Sawai et al. 2011). This suggests that *M. alexandrini* and Pacific *M. mola* are present in Japanese and Pacific Australia/New Zealand waters, respectively, predominantly as large adults, possibly outside the areas of reproduction for each species. The single Atlantic *M. mola* found in our study off Western Australia (Figure 3.3D) was relatively small (1.2 m). To date, very few Atlantic *M. mola* have been included in phylogenetic studies anywhere (D-loop: n = 3 [Yoshita et al. 2009, this study]; partial D-loop: n = 5 [Bass et al. 2005, Ahuir-Baraja et al. 2017]). One of these Atlantic *M. mola* specimens was sampled off the south coast of South Africa (Accession number AY940816), which together with our findings, suggests that this clade may have a range extension into the Indian Ocean. Further studies are needed to reveal how common the Atlantic *M. mola* clade is off Western Australia, and if the Atlantic and Pacific *M. mola* clades are restricted to the west and east coast of Australia, respectively, and if so, how the Great Australian Bight might act as a distribution barrier.

3.5.5 Implications for sunfish IUCN listing

Prior to this study, sunfish in both Australia and New Zealand were commonly "identified" as *M. mola*, or a mix of *M. mola* and *M. alexandrini*, with *Ma. lanceolatus* rarely mentioned. Moreover, prior to its recent description, *M. tecta* was mistaken for other *Mola* species (Table 3.2) (Nyegaard et al. 2018b [Chapter 2]; Table A3.1.1 in Appendix A3.1). Our results imply that the fisheries observer bycatch data collected in the ETBF and New Zealand longline fisheries and recorded predominantly as *M. mola* and Unspecified Molidae, consists mainly of three species of sunfish, *Ma. lanceolatus*, *M. alexandrini* and *M. tecta*. Consequently, these observer data should be used with caution for making inferences about trends in sunfish bycatch and the significance of their interactions with fisheries. Here, we used a simple CPUE analysis to investigate the annual CPUE trends in four fishing ground subareas, representing different marine climate zones, each presumably dominated by one species of sunfish. No discernible CPUE trends were found in either of the four areas, with large inter-annual fluctuations similar to sunfish bycatch CPUEs reported by Hahlbeck et al. (2017) in the Californian large-mesh drift gillnet fishery. Neither did our results show consistent patterns of increase or decrease over time, which indicates longline fishing pressure probably did not unduly influence the relative sunfish catch rates between 2001 and 2013 in Australia, and between 2000 and 2015 in New Zealand. However, a better understanding of the distribution of species and populations, their seasonal movements across the fishing grounds as well as fishing fleet behaviour, is needed for more robust conclusions.

On average, the annual mean observed CPUEs across the Australian (ETBF) and New Zealand longline fishing grounds between 2001 – 13 (< 0.3 sunfish.1,000 hooks^{-1}), were comparable to the annual mean CPUE in the South African longline fishery between 2000 – 03 (mean ± 1 SE = 0.17 ± 0.05 sunfish.1,000 hooks^{-1}; Petersen 2005). The South African longline fishery was cited by the IUCN as having very high sunfish bycatches, with an estimated 340,000 annual interactions (Sims et al. 2009b; Liu et al. 2015). However, this estimate appears to have been based on a misinterpretation of the CPUEs in Petersen (2005) by a factor of 1,000, i.e. 170 sunfish.1,000 hooks^{-1} (Sims et al. 2009b), instead of 0.17 sunfish.1,000 hooks^{-1} (Petersen 2005). The total sunfish interactions estimated by Petersen (2005) between 2000 and 2003 were < 500 year^{-1}, and of a similar magnitude to the total bycatch in the ETBF between 2010 and 2012 of < 500 year^{-1} (AFMA 2013) and not dissimilar to the estimated average total sunfish bycatch in New Zealand between 2012 and 2015 of 1,998 sunfish.year^{-1} (range 770 – 3,265) (MPI 2016b). While the high longline fishing pressure globally may have an impact on sunfish populations locally and/or globally, the comparatively modest total catches in both the Australian, New Zealand and South African fisheries suggest that the threat from longline fisheries may be less alarming than indicated in the IUCN listing (Liu et al. 2015).

Furthermore, the 2015 IUCN listing was restricted to *M. mola*, with *Ma. lanceolatus* classified as of ‘Least Concern‘ and *M. alexandrini* not assessed. Our results indicate that all the large species of ocean sunfish are likely to experience similar fishing pressure from longline activities (and presumably other fishing methods) within their distribution ranges. They also imply that investigations of sunfish species bycatch compositions are required to improve interpretation of bycatch trends and assessments of sunfish species vulnerability to fishing pressure, and to add to the understanding of sunfish species distributions globally.

3.5.6 Implications for sunfish research globally

Due to the nature of sunfish research, where data collection is often encumbered by the logistical constraints of observing and obtaining specimens for laboratory work, international collaboration is needed to piece together information on global species distributions, population units and connectivities, to ultimately assess the vulnerability of sunfish species to human pressures. Incorrect identification will cloud the compilation of global data sets and hinder overall progress in understanding these marine giants. The Molidae taxonomy is still undergoing review and further changes are likely in the future. We urge all researchers to explicitly state the basis for specimen identification, and to upload genetic sequences to open source platforms so that future re-evaluations are possible.

Appendix A3.1 Species identification of sunfish specimens (Genera *Mola* and *Masturus*, Family Molidae) from Australian and New Zealand natural history museum collections and other local sources

Abstract

This data-in-brief comprises a summary of sunfish specimens (Genera *Mola* and *Masturus*, Family Molidae, > 29 cm total length) from natural history museum collections and other sources (such as strandings) in Australia and New Zealand. Each specimen was evaluated morphologically and identified to lowest possible taxon based on recent advances in the understanding of the *Mola* taxonomy. References to phylogenetic analyses, where applicable, are included. The summary was collated in support of publication Giant jelly eaters on the line: species distribution and bycatch of three dominant sunfishes in the Southwest Pacific (Nyegaard et al. 2018a [Chapter 3]).

Specifications Table

Subject area	**Biology**
More specific subject area	Systematics, taxonomy, morphology
Type of data	Tables, images
How data was acquired	Specimen morphology was assessed in person or from photographs. Phylogenetic information was sourced from published literature.
Data format	Summary incl. metadata, images
Experimental factors	n/a
Experimental features	n/a
Data source location	Specimens are from the coastline and coastal waters of Australia and New Zealand (11°S – 168°S; 113°E – 179°E), held in museum collections in Adelaide (SAMA), Auckland (AIM), Brisbane (QM), Christchurch CMC), Dunedin (OMNZ), Hobart (TMAG), Melbourne (NMV), Perth (WAM), Sydney (AMS), Wellington (NMNZ), and London, UK (BMNH). Other material from stranding events in Australia and New Zealand is also included..
Data accessibility	Information on museum specimen held in Australian collections is available from the Online Zoological Collections of Australian Museums (OzCam) (http://ozcam.org.au). Genetic sequences are accessible from GenBank at the National Center for Biotechnology Information (NCBI) (*https://www.ncbi.nlm.nih.gov*) and/or the Barcode of Life Data (BOLD) System (*http://www.boldsystems.org*).

Value of the data

- Provides a comprehensive overview, including images, of sunfish specimens (genera *Mola* and *Masturus*) currently held in museum collections in Australia and New Zealand.
- Includes specimens not lodged with a museum, but for which samples are held by the authors.
- Resolves several errors in sunfish species identities of museum specimens, based on recent advances in the taxonomic understanding of the genus *Mola*.
- Provides information for researchers on where specimens and samples are held, for future research and collaboration.

Data

Table A3.1.1 lists material of ocean sunfishes (genera *Mola* and *Masturus* > 29 cm) held in Australian and New Zealand natural history museum collections. Table A3.1.2 lists specimens from Australian and New Zealand obtained from other sources, but which are not lodged with a natural history museum. The tables contain specimen detail, morphological assessment, Accession numbers for genetic sequences and references to phylogenetic studies, where applicable, and a verified or updated species identity. Tables A3.1.3 – A3.1.4 contain images, where available, of specimens in Table A3.1.1 – A3.1.2.

Experimental Design, Materials and Methods

Museum collections

The Auckland (AIM), Melbourne (NMV), Otago (OMNZ), Perth (WAM) and Wellington (NMNZ) museum collections were examined in person, while material from the Adelaide (SAMA), Brisbane (QM), Christchurch (CMC), Darwin (NTM), Hobart (TMAG), Sydney (AMS) and Whanganui (WRM) museums was examined via photographs and descriptions from examinations by museum staff or volunteers. The holotype of *Orthragoriscus ramsayi* (now *Mola alexandrini*) at the Natural History Museum in London (BMNH 1883.11.29.22), caught off New South Wales in 1882 (Giglioli, 1883; Whitley, 1931) and recently reviewed (Sawai et al., 2018), was also included for completeness. Museum codes are according to (Sabaj, 2013) except Otago Museum (OMNZ) and Whanganui Regional Museum (WRM).

Other material

Sunfish from other sources, such as strandings, were examined in person by one or both of the authors, or by volunteers. In the latter case, the morphology was also assessed from photographs by the authors.

Morphological assessment

Specimen morphology was assessed against relevant and recent literature (Fraser-Brunner 1951; Nyegaard et al. 2018b [Chapter 2]; Sawai et al. 2018). Each specimen was identified to the lowest possible taxon, noting that the two *Mola mola* (Linnaeus 1758) clades (Pacific vs Atlantic) (Ahuir-Baraja et al. 2017; Sawai et al. 2017; Nyegaard et al. 2018a [Chapter 3]) cannot be distinguished morphologically from the currently available literature (Sawai et al. 2018). For specimens examined from photographs, as many characters as possible were assessed, and a specimen identification assigned when a subjectively satisfactory combination of clear traits was available. Some of these specimens were also included in for the description of *Mola tecta* Nyegaard et al. 2017, and re-description of *Mola alexandrini* (Ranzani 1839) [formerly *Mola ramsayi* (Giglioli 1883)] (Nyegaard et al. 2018b; Sawai et al. 2018). Several specimens have also been included in various phylogenetic analyses elsewhere (Table A3.1.1 – A3.1.2).

The following taxonomic features were assessed:

- Protruding snout (SN) – present / absent
- Head bump (HB) – present / absent
- Chin bump (CB) – present / absent
- Dorso-lateral ridge (DLR) – swollen / absent
- Ventro-lateral ridge (VLR) – swollen / absent
- Smooth band back-fold (SBBF) – present / absent
- Clauvs shape (CS) – lobed, wavy / rounded / rounded with a small indent
- Number of ossicles (NOs)
- Paraxial ossicles (POs) – merged or separate
- Scale morphology (SM) – according to (Sawai et al. 2018)

Table A3.1.1 Whole museum specimens (> 29 cm), and other *Mola* spp. and *Masturus lanceolatus* material identifiable to species level, held in collections in Australia and New Zealand. Museum codes according to Sabaj (2016), except ABTC (Australian Biological Tissue Collection), OMNZ (Otago Museum) and WRM (Whanganui Regional Museum). State/country abbreviations are New South Wales (NSW), New Zealand (NZ), Northern Territory (NT), South Australia (SA), Tasmania (TAS), Victoria (VIC), Western Australia (WA), Queensland (QLD). Species identity established from morphology (**MORPH**), and/or phylogeny (**PHYL**) based on mtDNA D-loop and/or Cytochrome *c* oxidase 1 (CO1) loci. Taxonomic features assessed in person (**pers**), via photographs (**photo**) and/or X-rays (**xray**): protruding snout (**PS**), head bump (**HB**), chin bump (**CB**), dorso-lateral ridge (**DLR**), ventro-lateral ridge (**VLR**), clauvs shape (**CS**), smooth band back-fold (**SBBF**), number of ossicles (**NOs**), state of paraxial ossicles (**POs**), scale morphology (**SM**). Some features could not be assessed (**n/a**).

Museum registration number and specimen details	**Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.**	**Original museum ID**	**Species reassignment**
AIM MA29864, 51.1 cm TL, female, wet specimen, Poor Knights Islands, NZ (35.488°S 174.729°E[a]) 8 November 1969.	**MORPH (pers, xray)**: Nyegaard et al. (2018b).	*M. mola*	*M. tecta*
AIM MA30933, 102 cm TL, cast, likely from NZ waters.	**MORPH (pers)**: Nyegaard et al. (2018b).	*M. mola*	*M. tecta*
AIM MA30934, 211 cm TL, cast, likely from NZ waters.	**MORPH (pers)**: Sawai et al. (2018).	*M. mola*	*M. alexandrini*
AMS I.2742, 174 cm TL[b], mounted skin, Manly Beach, NSW (33.800°S 151.283°E), purchased, November 1882.	**MORPH (photo)**: **PS** present, **HB** & **CB** present, **DLR** & **VLR** n/a, **CS** rounded, **SBBF** n/a, **NO** n/a, **POs** n/a, **SM** n/a.	*M. mola*	*M. alexandrini*
AMS I.9412[c], 254 cm TL, mounted skin in poor condition, restored in 2012, Manly, NSW (33.800°S 151.283°E), beach cast, 16 December 1882 (1883 in Whitley 1931).	**MORPH (photo)**: **PS** present, **HB** & **CB** present (large), **DLR** & **VLR**: swollen, **CS** rounded, **SBBF** n/a**, NOs** n/a**, POs** n/a**, SM** n/a.	*M. mola*	*M. alexandrini*
AMS I.18215-001, 51.1 cm TL, wet specimen, East of Ulladulla, NSW (35.283°S 150.700°E), caught by FRV Kapala, 2 May 1973.	**MORPH (photo)**: **PS** absent, **HB** & **CB** + **DLR** & **VLR** absent (consistent with small specimen), **CS** rounded, **SBBF** absent, **NOs** >5 (large), **SM** consistent with *M. alexandrini* immature scale morphology on specimen NMNZ P.056071. **POs** n/a.	*M. ramsayi*	*M. alexandrini*

Museum registration number and specimen details	Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.	Original museum ID	Species reassignment
AMS I.25630-001, 60 cm TL, wet specimen, Crowdy Head, NSW (31.000°S 153.000°E), trawler, September 1985.	**MORPH (photo)**: **PS** absent, **HB** & **CB** + **DLR** & **VLR** absent (consistent with small specimen), **CS** rounded, **SBBF** absent, **NOs** >10 (large), **SM** consistent with *M. alexandrini* (Sawai et al. 2018). **POs** n/a.	*M. mola*	*M. alexandrini*
AMS I.38997-001, 250 cm TL, tissue & photographs, off Jervis Bay, NSW (35.05°S 150.733°E), caught on bow of MV "Goliath", 13 October 1998.	**MORPH (photo)**: **PS** absent, **HB** & **CB** + **DLR** & **VLR** present, **CS** rounded, **SBBF** absent. **NOs** n/a, **POs** n/a, **SM** n/a. **PHYL**: JNSW-2 in Yoshita et al. (2009) (D-loop: AB439109). Also in Ahuir-Baraja et al. (2017), Sawai et al. (2017) and Nyegaard et al. (2018a).	*M. mola*	*M. alexandrini*
AMS I.41536-001, 178 cm TL, tissue & photographs, Narrabeen Beach, NSW (35.716°S 151.300°E), found alive in shallow waters in poor condition (euthanized), 11 September 2002.	**MORPH (photo)**: **PS** absent, **HB** & **CB** absent, **DLR** short**, VLR** absent, **CS** rounded with indent, **SBBF** present, **NOs** 8[d]. **POs** n/a, **SM** n/a. **PHYL:** NNSW-1 in Yoshita et al. (2009) (D-loop: AB439108). Also in Ahuir-Baraja et al. (2017), Sawai et al. (2017) and Nyegaard et al. (2018a). Submission to BOLD by AMS (CO1 sequence ID: AMS174-08), phylogenetic analysis in Nyegaard et al. (2018b).	*M. mola*	*M. tecta*
AMS I.42801-001, 175 cm TL, tissue & photographs, Sussex Inlet, NSW (35.150°S 150.600°E), beach cast, 21 August 2003.	**MORPH (photo)**: **PS** present, **HB** & **CB** present (large), **DLR** & **VLR** slightly swollen (specimen not fresh), **CS** rounded, **SBBF** very faint, **NOs** >10. **POs** n/a, **SM** n/a. **PHYL:** SNSW-3 in Yoshita et al. (2009) (D-loop: AB439110). Also in Ahuir-Baraja et al. (2017), Sawai et al. (2017) and Nyegaard et al. (2018a).	*M. ramsayi*	*M. alexandrini*
AMS I.44396-001, 170 cm TL, tissue & photographs, near Robe, SA (37.150°S 139.750°E), beach cast, August 2007.	**MORPH (photo)**: **CS** with extension. **PHYL:** submission to BOLD by AMS (CO1 sequence ID: AMS124-08), phylogenetic analysis in Nyegaard et al. (2018b).	*Ma. lanceolatus*	*Ma. lanceolatus*
CMC F228, 42 cm TL, wet specimen, Scarborough Beach, Christchurch, South Island (*ca*. 43.566°S 172.761°E), beach cast, NZ, 13 July 1964	**MORPH (photo)**: presumably *M. alexandrini* from overall morphology, but insufficient characteristics visible on photograph to verify species identity.	*M. mola*	*Mola sp.*
CMC, no registration number available, mounted specimen.	Not assessed (no access).	*M. mola*	-

Museum registration number and specimen details	Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.	Original museum ID	Species reassignment
NMNZ P.001418, 60.2 cm TL, male, wet specimen, off Hawke's Bay, NZ (39.417°S 177.100°E), June 1952.	**MORPH (pers, xrays)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
NMNZ P.002629, 253.6 cm TL, cast (right side), Palliser Bay, NZ (*ca*. 41.442°S 175.008°E), 7 April 1930.	**MORPH (pers)**: Sawai et al. (2018).	*M. ramsayi*	*M. mola*
NMNZ P.002980, 64.6 cm TL, male, wet specimen, North Rona Bay, Eastbourne, Wellington Harbour, NZ (41.267°S 174.917°E), drag net, 30 November 1960.	**MORPH (pers)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
NMNZ P.005890, 49.9 cm TL, female, wet specimen, off Great Barrier Island, NZ (36.083°S 175.583°E), trawl 36–37 m, 25 June 1973.	**MORPH (pers, xrays)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
NMNZ P.006126, 64.8 cm TL, female, wet specimen, Oriental Bay, Wellington Harbour, NZ (41.290°S 174.793°E), beach cast, 8 Dec 1974[a].	**MORPH (pers, xrays)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
NMNZ P.006345, 38.8 cm TL, male, wet specimen, off Te Kaha, Bay of Plenty, North Island, NZ (37.650°S 177.517°E), purse seine, 2 March 1976.	**MORPH (pers, xrays)**: Sawai et al. (2018).	*M. ramsayi*	*M. alexandrini*
NMNZ P.009864, 121.6 cm TL, wet specimen, North of Cape Brett, North Auckland, NZ (34.917°S 174.567°E), purse sein, 27 January 1981.	**MORPH (pers)**: **CS** with extension.	*Ma. lanceolatus*	*Ma. lanceolatus*
NMNZ P.009887, 38.5 cm TL, male, wet specimen, off North Cape, North Auckland, NZ (35.217°S 172.467°E), 5 February 1980.	**MORPH (pers, xrays)**: Sawai et al. (2018).	*M. ramsayi*	*M. alexandrini*
NMNZ P.033995, 57.2 cm TL, male, wet specimen, Bay of Plenty, Opotiki Beach, NZ (37.750°S 177.333°E), November 1996.	**MORPH (pers, xrays)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
NMNZ P.034187, 79.7 cm TL, male, wet specimen, Bay of Plenty, surf at Opotiki, NZ (37.750°S 177.333°E).	**MORPH (pers, xrays)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
NMNZ P.034217, 69.8 cm TL, male, wet specimen, Bay of Plenty, Opotiki Beach, NZ (37.733°S 177.333°E), December 1996.	**MORPH (pers, xrays)**: Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*

Museum registration number and specimen details	Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.	Original museum ID	Species reassignment
NMNZ P.034449, 51.5 cm TL, female, wet specimen, Southern Colville Ridge, Bay of Plenty, NZ (36.392°S 176.850°E), surface longline, 17 May 1997.	**MORPH (pers, xrays):** Sawai et al. (2018).	*M. ramsayi*	*M. alexandrini*
NMNZ P.036964, 45.3 cm TL, female, wet specimen, Off Mahia Peninsula, Hawke's Bay, NZ (39.083°S 178.883°E), 13 May 1999.	**MORPH (pers, xrays)**: Sawai et al. (2018).	*M. ramsayi*	*M. alexandrini*
NMNZ P.056054, 212.7 cm TL, male, wet specimen, Omaha Beach, north of Tawharanui Peninsula, North Auckland, NZ (36.350°S 174.785°E), beach cast, 14 May 2013.	**MORPH (photo)**: **CS** with extension.	*Ma. lanceolatus*	*Ma. lanceolatus*
NMNZ P.056071, 29.3 cm TL, male, wet specimen (tissue **P.056071/TS1**), Off Raglan Harbour, Taranaki, NZ (37.728°S 174.168°E), trawl, 28 December 2012.	**MORPH (pers)**: Sawai et al. (2018). **PHYL:** Nyegaard et al. (2018b) (D-loop: MF158140; CO1: MF158118). Also in Nyegaard et al. (2018a) (D-loop).	*M. ramsayi*	*M. alexandrini*
NMNZ P.057679[e], holotype, 101.1 cm TL, wet specimen (tissue **P.057679/TS3**), male, North Taranaki Bight, west coast of North Island, NZ (38.425°S 174.150°E), trawl, 78 m, 25 December 2015.	**MORPH (pers, xrays)**: Nyegaard et al. (2018b) **PHYL**: Nyegaard et al. (2018b) (D-loop: MF158147; CO1: MF158119). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NMV 32054, large, mounted skeleton, Port Phillip Bay, Hobsons Bay, VIC (37.850°S 144.930°E), 1879.	Not assessed (no access).	*M. mola*	-
NMV A 18725, 90.5 cm TL, wet specimen, Port Phillip Bay, VIC (37.8670°S 144.817°E), hook and line, 28 August 1995.	**MORPH (pers)**: Nyegaard et al. (2018b).	*M. mola*	*M. tecta*
NMV A 26565-001, 78.5 cm TL, wet specimen & tissue, 13th beach near Barwon Heads, VIC (38.286°S 144.456°E), beach cast, 30 April 2009.	**MORPH (pers)**: Nyegaard et al. (2018b). **PHYL:** Nyegaard et al. (2018a) (D-loop: MG254032)	*M. ramsayI*	*M. tecta*
NMV A 30811-001, *ca*. 85 cm TL, tissue & photographs, Port Augusta power station, SA (*ca*. 32.545°S 137.788°E), beach cast, 23 September 2008.	**MORPH (photo, xray): PS** absent, **HB** & **CB** absent**, VLR** absent, **DLR** short, **CS** rounded with indent, **SBBF** present, **NOs** 6, **POs** separate. **PHYL** Nyegaard et al (2018a) (D-loop: MG254031).	*Mola sp.*	*M. tecta*

Museum registration number and specimen details	**Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.**	**Original museum ID**	**Species reassignment**
NMV A 25071-001, *ca*. 200 cm TL, skin sample & photographs (**NMV A 25071-002**), tissue (**NMV Z 10859**), Bunurong Marine Park (38.651°S 145.693°E), beach cast, 10 April 2003.	**MORPH (photo): PS** present (small), **HB** & **CB** present (small)**, DLR** & **VLR** swollen, **CS** n/a, **SBBF** n/a, **NOs** n/a, **POs** n/a,**SM** n/a. **PHYL:** Nyegaard et al. (2018a) (D-loop: MG254033). Submission to BOLD by NMV (CO1 sequence ID: FMVIC396-08), phylogenetic analysis in Nyegaard et al. (2018b).	*Mola sp.*	*M. mola* (Pacific clade)
NMV A 31759-001, 170 cm, parts & photographs, tissue (**NMV ZZ 61327**), Lake Tyers mouth, East Gippsland, VIC (37.855°S 148.101°E), beach cast, 23 March 2017.	**MORPH (photo):** Body shape consistent with *Ma. lanceolatus*, but clavus damaged (**CS** n/a). **PHYL:** Nyegaard et al. (2018a) (D-loop: MG254034).	*Ma. lanceolatus*	*Ma. lanceolatus*
NTM S.15520-001, 106.5 cm TL, wet specimen & tissue, Cobourg Peninsula, NT (11.117°S 132.150°E), beach cast, 7 February 2003.	**MORPH (photo): CS** with extension. **PHYL:** Yoshita et al. (2009) (D-loop: AB439120). Also in Nyegaard et al. (2018a)	*M. ramsayi*	*Ma. lanceolatus*
OMNZ VT3248, 242 cm TL, cast (left side) from fresh specimen, Otago Harbour, Dunedin, NZ (45.883°S 170.508°E[a]), 1961.	**MORPH (pers):** Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
OMNZ VT3249, 78 cm TL, cast (right side) from fresh specimen, Kaka Point, Clucha District, NZ, (46.367°S 169.733°E), beach cast, 7 March 1963.	**MORPH (pers):** Nyegaard et al. (2018b).	*M. ramsayi*	*M. tecta*
OMNZ X2017.18, 58 cm TL, parts & photographs, Aramoana, NZ (45.766°S 170.696°E), beach cast, 9 July 2015. Tissue sample held by MN.	**MORPH (photo): PS** absent, **HB** & **CB + DLR** & **VLR** absent (consistent with small specimen), **CS**: rounded, **SBBF** absent, **NOs** >7 (large). **SM** n/a, **POs** n/a. **PHYL:** Nyegaard et al. (2018b) (D-loop: MF158135; CO1: MF158129). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp.*	*M. alexandrini*
OMNZ X2017.19, 169 cm TL, female, parts & photographs, Aramoana saltmarshes, Dunedin, NZ (45.782°S 170.711°E[a]), beach cast, 18 January 2017. Tissue sample held by MN.	**MORPH (photo)**: Nyegaard et al. (2018b) **PHYL:** Nyegaard et al. (2018b) (D-loop: MF158136; CO1: MF158130). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp.*	*M. tecta*
QM I10163, 200 cm TL, cast, Burleigh Heads, Queensland, Australia (28.083°S 153.450°E), beach cast, 18 November 1968.	**MORPH (photo): PS** present (small), **HB** & **CB** present, **DLR** &**VLR** swollen, **CS** rounded, **SBBF** absent. **NOs** n/a, **POs** n/a**, SM** n/a.	*M. mola*	*M. alexandrini*
SAMA AMSTAC1924, presumably wet specimen, Brown's Bay, SA (38.048°S 140.838°E), beach cast, 19 August 1982.	Not assessed (no access).	*M. ramsayi*	-

Museum registration number and specimen details	Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.	Original museum ID	Species reassignment
SAMA F243, *ca*. 134 cm TL, cast (left side), likely from SA, 1914	**MORPH (photo)**: **PS** present, **HB** & **CB** present, **DLR** & **VLR** n/a, **CS** rounded, **SBBF** absent, **NO** n/a (features appear to have been lost during cast preparation), **POs** n/a, **SM** n/a.	*M. mola*	*M. alexandrini*
SAMA F6046, presumably wet specimen, Port River, Adelaide, SA (34.800°S 138. 517°E), 13 November 1982.	Not assessed (no access).	*M. ramsayi*	-
SAMA F7542, *ca*. 90 cm TL, wet specimen & tissue (**ABTC21528**), Spencer gulf, SA (34.780°S 138.480°E), 27 June 1994[f].	**MORPH (photo)**: Nyegaard et al. (2018b) **PHYL:** Nyegaard et al. (2018b) (D-loop: MF158148). Also in Nyegaard et al. (2018a)	*M. ramsayi*	*M. tecta*
SAMA F8085, presumably wet specimen, Port Augusta, SA (32.500°S 137.783°E), beach cast, July 1996.	Not assessed (no access).	*Mola sp.*	-
SAMA F9303, presumably wet specimen, Victor Harbour, SA (*ca*. 35.549°S 138.627°E), November 1999.	Not assessed (no access).	*M. mola*	-
SAMA F3316 – F3319, parts, Spencer Gulf, (SA 35.717°S 137.950°E), 31 July 1965.	Not assessed (no access).	*Ma. lanceolatus*	-
TMAG D3693, *ca*. 200 cm TL, photographs, White Beach, Tasmania (43.120°S 147.740°E), beach cast, 2003.	**MORPH (photo): PS** present (small), **HB** & **CB** present, **DLR** & **VLR** swollen, **CS** rounded, **SBBF** absent. **NO** n/a, **POs** n/a, **SM** n/a.	*M. mola*	*M. alexandrini*
TMAG D3885, *ca*. 50 cm TL, frozen specimen, Randalls Bay, TAS (*ca*. 43.243 E 147.137 E), 2010.	**MORPH (photo)**: presumably *M. alexandrini* from overall morphology, but insufficient characteristics visible on photograph to verify species identity. No access to specimen.	*Mola sp.*	*Mola sp.*
TMAG D3912, 150 cm TL, tissue & photographs, Lindisfarne, TAS (42.850°S 147.333°E), beach cast, 12 December 2014.	**MORPH (photo)**: Nyegaard et al. (2018b) **PHYL**: Nyegaard et al. (2018b) (D-loop: MF158149). Also in Nyegaard et al. (2018a)	*Mola sp.*	*M. tecta*
WAM P.33481-001, 138 cm TL, wet specimen, Augusta, WA (34.190°S 115.100°E), beach cast, August 2010.	**MORPH (pers):** present, **HB** absent, **CB** present (small), **DLR** short, **VLR** absent, **CS** rounded, **SBBF** present (very faint), **NOs** 11-12 (large), **SM** consistent with *M. alexandrini*. **POs** n/a.	*M. ramsayi*	*M. alexandrini*
WRM 1895.39, >200 cm TL, mounted skin (poor condition) Napier Harbour, NZ (*ca*. 39.482°S 176.894°E), 'captured', May 1895.	**MORPH (photo): PS** absent, **HB** & **CB + DLR** & **VLR** absent (features likely lost during extensive preparation and repair	*M. Mola*	*M. alexandrini*

Museum registration number and specimen details	Basis for specimen identification in this study; DNA sequence accession numbers in parentheses.	Original museum ID	Species reassignment
	of specimen in poor condition), **CS** rounded, **NO** >10 (large). **SM+POs** n/a.		
BMNH 1883.11.29.22, 229.1 cm TL, holotype of *Orthragoriscus ramsayi* (Giglioli 1883), mounted skin restored in 2016, Darling Harbour, NSW (*ca*. 33.85°S 151.20°E) beach cast, 1882.	**MORPH (pers):** Sawai et al. (2018)	*M. ramsayi*	*M. alexandrini*

[a] Updated since Nyegaard et al. (2018b)

[b] Total Length (TL) from Whitley (1931)

[c] Specimen recently found by AMS without a label in a storage facility; based on extensive research of old records AMS conclude this is most likely AMS I.9412, or alternatively AMS I.5312, which stranded *ca*. 1871-1874, probably in Manly Harbour (M. McGrouther, AMS, pers. comm. 2017), and was highly likely also a *M. alexandrini* (Whitley 1931).

[d] Number of ossicles ('NO') from Voegelnest (2003)

[e] Included in sea surface temperature analysis in Nyegaard et al. (2018a)

[f] Collection year on current specimen label is 1989, but the collection authority considers this an error (R Foster, SAMA, pers comm 2017).

Table A3.1.2 Australian and New Zealand Molidae material obtained from strandings and other sources. State/country abbreviations are New South Wales (NSW), New Zealand (NZ) and Western Australia (WA). Species identity established from morphology (**MORPH**), and/or phylogeny (**PHYL**) based on mtDNA D-loop and/or Cytochrome *c* oxidase 1 (CO1) loci. Taxonomic features assessed in person by one or both authors (**pers**) or via photographs (**photo**).

Sample number and specimen detail	**Basis for specimen identification; DNA sequence accession numbers in parentheses.**	**Species ID by sampler**	**Species ID in this study**
NZ01, 212 cm TL, Otago Harbour, NZ (45.817°S 170.617°E), beach cast, 18 February 2015. Specimen not retained, tissue held by MN.	MORPH (photo): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158134). Also in Nyegaard et al. 2018a.	*Mola sp. nov*	*M. tecta*
NZ07, 100 cm TL, Banks Peninsula, NZ (43.833°S 172.667°E), beach cast, 30 April 2014. Specimen not retained, tissue held by MN.	MORPH (photo): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158137; CO1: MF158120). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ08, 151 cm TL, Banks Peninsula, NZ (43.833°S 172.667°E), beach cast, 30 April 2014. Specimen not retained, tissue held by MN.	MORPH (photo): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158138; CO1: MF158121). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ09, 193 cm TL, Banks Peninsula, NZ (43.833°S 172.667°E), beach cast, 30 April 2014. Specimen not retained, tissue held by MN.	MORPH (photo): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158139; CO1: MF158122). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ12, 155 cm TL, female, Birdling's Flat, Banks Peninsula, NZ (43.817°S 172.700°E), beach cast, 10 May 2014. Specimen not retained, tissue held by MN, skin sample and clavus held by ES.	MORPH (pers): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158141; CO1: MF158123). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ14, 170 cm TL, near Birdling's Flat, Banks Peninsula, NZ (43.833°S 172.667°E), beach cast, 14 December 2015. Specimen not retained, tissue held by MN.	MORPH (photo): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158142; CO1: MF158124). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ16, 261 cm TL, Kaka Point, South Otago, South Island, NZ (46.417°S 169.783°E), beach cast, 9 October 2011. Specimen not retained, tissue held by MN.	PHYL: Nyegaard et al. (2018b) (D-loop: MF158143; CO1: MF158125). Also in Nyegaard et al. (2018a) (D-loop).	*M. mola*	*M. mola* (Pacific clade)

NZ17[a], 81 cm TL, male, west of South Island, NZ (41.533°S 170.933°E), purse seine, 12 April 2014. Specimen not retained, tissue held by MN, skin sample and clavus held by ES.	MORPH (pers): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158144; CO1: MF158126). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ18[a,b], 65 cm TL, female, east of North Island, NZ (39.783°S 178.417°E), longline, 17 May 2014. Specimen not retained, genetic sample held by MN, clavus held by ES.	MORPH (pers): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158145 & CO1: MF158127). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
NZ19[a], 69 cm TL, male, east of North Island, NZ (35.150°S 176.050°E), longline, 10 August 2014. Specimen not retained, genetic sample held by MN, clavus held by ES.	MORPH (pers): Nyegaard et al. (2018b) PHYL: Nyegaard et al. (2018b) (D-loop: MF158146; CO1: MF158128). Also in Nyegaard et al. (2018a) (D-loop).	*Mola sp. nov.*	*M. tecta*
WA41[a], near Bremer Canyon, WA (*ca*. 34.667°S 120.133°E), propeller strike, 22 April 2015. Specimen not retained, tissue held by MN.	PHYL: Nyegaard et al. (2018a) (D-loop: MG254030).	*Mola sp.*	*M. alexandrini*
NNZ-1b, *ca*. 100 cm TL, off North NZ (ca. 30.000°S 175.000°E), longline, July 2007. Specimen not retained, tissue held by ES.	PHYL: Sawai et al. (2018) (Electronic Supplementary Material Table S1) (D-loop: LC271189). Also in Nyegaard et al. (2018a).	Molidae	*M. alexandrini*

[a] Included in sea surface temperature analysis in Nyegaard et al. (2018a)

[b] Coordinates updated since Nyegaard et al. (2018b)

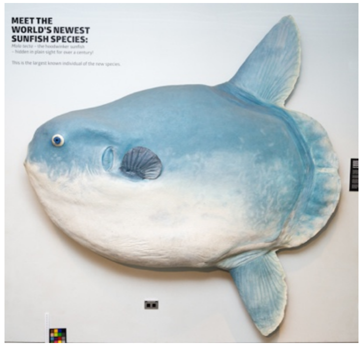
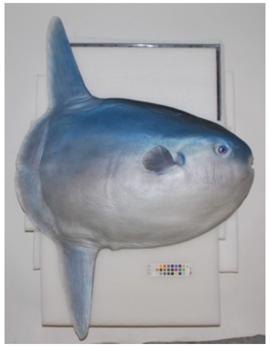
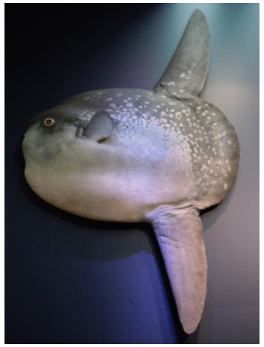
Table A3.1.3 Photographs of museum specimens (Table 1). No photographs were available for specimens CMC (mounted specimen, no registration number), NNZ-1b, SAMA AMSTAC1924, NMV 32054, SAMA F6046, SAMA F8085, SAMA F9303, SAMA F3316 – F3319.

AIM MA29864  Photo by Tom Trnski, AIM	**AIM MA30933**  Photo by Tom Trnski, AIM	**AIM MA30934**  Photo by Tom Trnski, AIM	**AMS I.2742**  Photo by Mark McGrouther, AMS
AMS I.9412  Photo by Stuart Humphreys, AMS	**AMS I.18215-001**  Photo by Joanna Browne	**AMS I.25630-001**  Photo by Joanna Browne	**AMS I.38997-001**  Photo by Paul Ovenden, AMS

AMS I.41536-001  Photo by Kerryn Parkinson, AMS	**AMS I.42801-001**  Photo by NSW DPI fisheries, Shoalhaven office	**AMS I.44396-001**  Photo by Nicole Slater	**CMC F228**  Photo provided by CMC
NMNZ P.001418  Photo by Etsuro Sawai	**NMNZ P.002629**  Photo by Marianne Nyegaard	**NMNZ P.002980**  Photo by Marianne Nyegaard	**NMNZ P.005890**  Photo by Etsuro Sawai

NMNZ P.006126  Photo by Marianne Nyegaard	**NMNZ P.006345**  Photo by Etsuro Sawai	**NMNZ P.009864**  Photo by Etsuro Sawai	**NMNZ P.009887**  Photo by Etsuro Sawai
NMNZ P.033995  Photo by Marianne Nyegaard	**NMNZ P.034187**  Photo by Etsuro Sawai	**NMNZ P.034217**  Photo by Etsuro Sawai	**NMNZ P.034449**  Photo by Etsuro Sawai

NMNZ P.036964  Photo by Etsuro Sawai	**NMNZ P.056054**  Photo by Jean-Claude Stahl, NMNZ	**NMNZ P.056071**  Photo by Andrew Stewart, NMNZ	**NMNZ P.057679**  Photo by Carl Struthers, NMNZ
NMV A18725  Photo by Marianne Nyegaard	**NMV A26565-001**  Photo by Marianne Nyegaard	**NMV A30811-001**  Photo by Richard Saunders	**NMV A25071-001**  Photo provided by NMV

NMV A31759-001	**NTM S.15520-001**	**OMNZ VT3248**	**OMNZ VT3249**
			
Photo provided by NMV	Photo by Gavin Dally, NTM	Photo by Kane Fleury, OMNZ	Photo by Kane Fleury, OMNZ
OMNZ X2017.18	**OMNZ X2017.19**	**QM I10163**	**SAMA F243**
			
Photo by Emma Burns, OMNZ	Photo by Kane Fleury, OMNZ	Photo by Jeff Johnson, QM	Photo by Julie Mattiske

SAMA F7542  Photo by Julie Mattiske	**TMAG D3693**  Photo by Pat Graham	**TMAG D3885**  Photo provided by TMAG	**TMAG D3912**  Photo by Kathryn Medlock, TMAG
WAM P.33481-001  Photo by Gilbert Stockman	**WRM 1895.39**  Photo provided by WRM	**BMNH 1883.11.29.22**  Photo by Etsuro Sawai	

Table A3.1.4 Photographs of specimens from other sources (Table 2).

NZ01	**NZ07**	**NZ08**	**NZ09**
			
Photo by Jean McKinnon	Photo by Ken Logan	Photo by Ken Logan	Photo by Ken Logan
NZ12	**NZ14**	**NZ16**	**NZ17**
			
Photo by Marianne Nyegaard	Photo by Roscoe Le Compte	Photo by Cheryl Pullar, DOC[a]	Photo by Marianne Nyegaard

NZ18	**NZ19**	**WA41**	
			
Photo by Marianne Nyegaard	Photo by Marianne Nyegaard	Photo provided by DPIRD[b]	

[a] New Zealand Department of Conservation Te Papa Atawhai

[b] Department of Primary Industries and Regional Development, Government of Western Australia (Fisheries division).

Appendix A3.2 Supplementary information to Chapter 3

Table A2.3.1 Biopsy samples from sunfish caught as bycatch in the longline fishery in Australia and New Zealand, collected by fisheries observers from the Australian Fisheries Management Authority, and the New Zealand Ministry of Primary Industries. Area codes are Eastern Tuna and Billfish Fishery (ETBF), Western Tuna and Billfish Fishery (WTBF), New Zealand (NZ).

Sample name	**Sampling date (DD/MM/YYYY)**	**e-TL (cm)**	**Genetic species clade**	**Area**	**Latitude (South)**	**Longitude (East)**	**Accession Number**
B02	24/08/2013	130	*Masturus lanceolatus*	ETBF	23.267	156.067	MG253927
B04	24/08/2013	130	*Masturus lanceolatus*	ETBF	23.767	156.433	MG253928
B05	23/08/2013	115	*Mola alexandrini*	ETBF	23.683	156.983	MG253929
C01	08/03/2013	120	*Masturus lanceolatus*	ETBF	35.653	151.425	MG253930
C02	09/03/2013	150	*Mola alexandrini*	ETBF	36.159	151.448	MG253931
D01	20/11/2013	165	*Mola alexandrini*	ETBF	24.535	157.597	MG253932
D02	25/07/2013	120	*Mola alexandrini*	ETBF	35.801	151.214	MG253933
D03	19/11/2013	160	*Masturus lanceolatus*	ETBF	24.465	157.707	MG253934
D04	18/11/2013	160	*Masturus lanceolatus*	ETBF	24.763	157.802	MG253935
D05	17/11/2013	130	*Masturus lanceolatus*	ETBF	24.653	157.886	MG253936
D06	17/11/2013	150	*Masturus lanceolatus*	ETBF	24.637	157.885	MG253937
D07	17/11/2013	170	*Masturus lanceolatus*	ETBF	24.697	157.765	MG253938
D08	19/11/2013	160	*Masturus lanceolatus*	ETBF	24.73	157.762	MG253939
D09	10/02/2014	100	*Masturus lanceolatus*	ETBF	24.333	155.633	MG253940
D10	11/02/2014	80	*Masturus lanceolatus*	ETBF	24.206	155.282	MG253941
D11	11/02/2014	150	*Masturus lanceolatus*	ETBF	24.206	155.282	MG253942
D12	09/02/2014	200	*Mola alexandrini*	ETBF	20.4	156.3	MG253943
D13	10/02/2014	100	*Masturus lanceolatus*	ETBF	24.333	155.633	MG253944
D15	06/10/2013	100	*Mola alexandrini*	ETBF	35.492	150.91	MG253945
E01	18/05/2013	150	*Masturus lanceolatus*	ETBF	26.286	155.304	MG253946
E02	18/05/2013	98	*Masturus lanceolatus*	ETBF	25.874	155.948	MG253947
E03	11/09/2013	80	*Masturus lanceolatus*	ETBF	25.544	156.4	MG253948
E06	23/09/2013	100	*Mola alexandrini*	ETBF	29.133	157.55	MG253949
E07	23/09/2013	100	*Masturus lanceolatus*	ETBF	29.1	157.2	MG253950
E08	24/09/2013	100	*Mola alexandrini*	ETBF	29.133	157.167	MG253951
E09	24/09/2013	100	*Mola alexandrini*	ETBF	29.133	157.167	MG253952
E10	16/11/2013	100	*Masturus lanceolatus*	ETBF	27.15	156.583	MG253953
E11	16/11/2013	100	*Masturus lanceolatus*	ETBF	27.15	156.583	MG253954
E12	16/11/2013	100	*Masturus lanceolatus*	ETBF	27.15	156.583	MG253955
E13	16/11/2013	100	*Masturus lanceolatus*	ETBF	27.15	156.583	MG253956
E14	16/11/2013	100	*Masturus lanceolatus*	ETBF	27.15	156.583	MG253957
E15	20/11/2013	100	*Masturus lanceolatus*	ETBF	27.033	154.8	MG253958
F01	09/02/2013	90	*Masturus lanceolatus*	ETBF	28.458	154.014	MG253959
F02	21/04/2013	130	*Mola alexandrini*	ETBF	21.595	154.851	MG253960
F03	22/04/2013	90	*Masturus lanceolatus*	ETBF	21.508	154.756	MG253961

Sample name	Sampling date (DD/MM/YYYY)	e-TL (cm)	Genetic species clade	Area	Latitude (South)	Longitude (East)	Accession Number
F04	23/04/2013	160	*Masturus lanceolatus*	ETBF	21.377	155.22	MG253962
F05	25/05/2013	110	*Masturus lanceolatus*	ETBF	19.667	153.633	MG253963
F06	27/05/2013	130	*Masturus lanceolatus*	ETBF	19.167	153	MG253964
F07	17/09/2013	120	*Mola alexandrini*	ETBF	25.424	155.094	MG253965
F08	30/05/2013	85	*Masturus lanceolatus*	ETBF	18.954	152.852	MG253966
F09	15/02/2014	180	*Masturus lanceolatus*	ETBF	28.731	158.223	MG253967
F10	18/09/2013	122	*Masturus lanceolatus*	ETBF	25.266	155.188	MG253968
F11	15/02/2014	140	*Masturus lanceolatus*	ETBF	28.738	158.364	MG253969
F12	19/09/2013	110	*Masturus lanceolatus*	ETBF	25.411	155.054	MG253970
F13	19/09/2013	103	*Masturus lanceolatus*	ETBF	25.488	155.22	MG253971
F14	15/09/2013	120	*Masturus lanceolatus*	ETBF	25.766	155.029	MG253972
F15	16/09/2013	130	*Mola alexandrini*	ETBF	25.318	154.987	MG253973
G01	20/08/2013	100	*Mola alexandrini*	NZ	31.667	172.117	MG253974
G02	21/08/2013	160	*Mola alexandrini*	NZ	31.217	172.6	MG253975
G03	22/08/2013	150	*Mola alexandrini*	NZ	31.133	172.467	MG253976
G04	23/08/2013	170	*Mola alexandrini*	NZ	31.933	171.933	MG253977
G06	08/07/2013	76	*Mola tecta*	NZ	36.500	178.400	MF158131[a]
G05	21/07/2013	90	*Mola tecta*	NZ	36.5	177.9	MG253978
G07	08/07/2013	62	*Mola tecta*	NZ	36.5	177.75	MG253979
G08	21/07/2013	90	*Mola alexandrini*	NZ	36.533	177.833	MG253980
G09	21/07/2013	50	*Mola tecta*	NZ	36.533	177.833	MG253984
G10	27/07/2013	120	*Mola alexandrini*	NZ	35.667	176.883	MF158132[a]
G11	24/08/2013	140	*Mola alexandrini*	NZ	31.983	171.967	MG253981
G12	26/08/2013	150	*Mola tecta*	NZ	32.517	172.017	MG253985
H01	07/09/2013	120	*Mola alexandrini*	NZ	32.137	171.208	MG253982
H02	26/07/2013	120	*Mola tecta*	NZ	36.267	177.167	MG253983
H03	09/09/2013	140	*Mola alexandrini*	NZ	32.651	172.52	MG253986
H04	09/09/2013	120	*Mola alexandrini*	NZ	32.569	172.353	MF158133[a]
H05	12/09/2013	81	*Mola alexandrini*	NZ	35.42	174.243	MG253988
H06	17/07/2013	60	*Mola tecta*	NZ	39.1	178.667	MG253987
H07	31/10/2013	130	*Mola alexandrini*	NZ	33.584	173.903	MG253989
H08	01/11/2013	120	*Mola alexandrini*	NZ	34.039	174.262	MG253990
H09	02/11/2013	100	*Mola alexandrini*	NZ	33.704	173.912	MG253991
H10	02/11/2013	90	*Mola alexandrini*	NZ	33.934	173.912	MG253992
H11	03/11/2013	100	*Mola alexandrini*	NZ	33.809	174.108	MG253993
H12	03/11/2013	150	*Mola alexandrini*	NZ	33.938	173.933	MG253994
H18	14/07/2014	130	*Mola alexandrini*	NZ	37.15	178.517	MG253995
H21	11/08/2014	175	*Mola alexandrini*	NZ	35.267	176.067	MG253996
H22	11/08/2014	150	*Mola tecta*	NZ	35.2	176	MG253997
H25	23/04/2014	140	*Mola alexandrini*	NZ	36.85	177.35	MG253998
H26	24/04/2014	130	*Mola alexandrini*	NZ	36.383	176.633	MG253999
H27	25/04/2014	100	*Mola alexandrini*	NZ	36.383	177	MG254000
K01	11/02/2014	130	*Masturus lanceolatus*	ETBF	24.483	155.167	MG254001
K03	08/02/2014	150	*Masturus lanceolatus*	ETBF	24.333	156.667	MG254002
K09	13/02/2014	180	*Mola alexandrini*	ETBF	26.883	158.357	MG254003

Sample name	Sampling date (DD/MM/YYYY)	e-TL (cm)	Genetic species clade	Area	Latitude (South)	Longitude (East)	Accession Number
K14	12/02/2014	200	*Masturus lanceolatus*	ETBF	29	158.128	MG254004
K15	09/02/2014	130	*Masturus lanceolatus*	ETBF	24.3	156.45	MG254005
K16	09/02/2014	150	*Masturus lanceolatus*	ETBF	24.333	156.35	MG254006
K18	11/02/2014	120	*Masturus lanceolatus*	ETBF	29.032	158.303	MG254007
K20	13/02/2014	150	*Masturus lanceolatus*	ETBF	26.885	158.357	MG254008
K21	10/02/2014	100	*Masturus lanceolatus*	ETBF	24.333	155.633	MG254009
K22	18/02/2014	120	*Masturus lanceolatus*	ETBF	29.013	159.008	MG254010
K23	18/02/2014	220	*Mola alexandrini*	ETBF	29.034	158.009	MG254011
K24	16/02/2014	130	*Masturus lanceolatus*	ETBF	28.601	158.527	MG254012
K33	20/02/2014	150	*Masturus lanceolatus*	ETBF	26.527	158.165	MG254013
K36	12/02/2014	180	*Masturus lanceolatus*	ETBF	29.034	158.244	MG254014
K39	18/02/2014	130	*Masturus lanceolatus*	ETBF	29.024	158.082	MG254015
K40	16/02/2014	150	*Masturus lanceolatus*	ETBF	28.585	158.47	MG254016
M01	25/04/2014	120	*Mola alexandrini*	WTBF	33.45	113.117	MG254017
M02	01/05/2014	90	*Mola alexandrini*	WTBF	29.783	112.1	MG254018
M10	29/05/2014	150	*Masturus lanceolatus*	WTBF	31.467	114.65	MG254019
M12	12/04/2014	130	*Mola alexandrini*	WTBF	34.533	112.35	MG254020
M14	03/05/2014	120	*Mola mola* (Atlantic clade)	WTBF	30.833	111.417	MG254021
M15	15/05/2014	200	*Mola alexandrini*	WTBF	31.717	114.033	MG254022
M16	25/04/2014	130	*Mola alexandrini*	WTBF	33.383	113.483	MG254023
M17	25/05/2014	140	*Mola alexandrini*	WTBF	29.967	114.017	MG254024
M18	25/05/2014	190	*Mola alexandrini*	WTBF	29.95	113.883	MG254025
M19	23/04/2014	50	*Mola alexandrini*	WTBF	34.533	113.9	MG254026
M20	14/04/2014	100	*Mola alexandrini*	WTBF	33.575	112.6	MG254027
M21	15/04/2014	100	*Mola alexandrini*	WTBF	34.167	113.117	MG254028
M48	21/09/2016	ND	*Mola alexandrini*	WTBF	31.5	112.86	MG254029

e-TL: estimated total length.

[a] First reported in Nyegaard et al. (2018b) [Chapter 2].

Chapter 4 Upwelling chasers or thermal regulators? Seasonality of the tourism-targeted sunfish (*Mola alexandrini*) in the dynamic Lombok Strait, Indonesia

4.1 Abstract

The iconic Bali sunfish, occurring seasonally on the Bali and Nusa Penida reefs in the Lombok Strait, Indonesia, is an important drawcard for the local SCUBA diver tourism industry. Diver crowding, however, is of growing concern, but a paucity of information on the Bali sunfish hinders development of management plans. Here, we aim to establish if temperature is the likely main driver of the Bali sunfish seasonality, as is anecdotally thought. We achieved this by first verifying the species as *Mola alexandrini* genetically (n = 3) and morphologically (n > 750). We then compared mean monthly sunfish encounter rates during dive operator tours (n = 4,592 dives) with seasonal changes in two major Lombok Strait oceanographic features, established from satellite sea surface temperature (SST) and at a finer scale, from *in situ* temperature loggers at four sites along a 14 km stretch of coast of Nusa Penida. We found a ~1 month lag between the 2015 sunfish season (August – October/November), and the seasonal drop in SST (July – October) associated both with an increase in the Indonesian Through Flow, as well as seasonal cold-water upwelling along the Bali southern coast. Eight *M. alexandrini* were tagged with Wildlife Computer SPLASH tags yielding a combined 287 tracking days between 21 August and 5 November 2015, with five tags providing data for > 40 days each. Combined they revealed a high affinity by four fish to the very dynamic southern and eastern Lombok Straight during the sunfish season, although at least one other fish left the area. The fish generally undertook daytime dives (at times > 500 m) to cold temperatures (generally < 15°C), presumably to forage. The tags revealed a high thermal tolerance for *M. alexandrini*, including the 27 – 29 °C range, well within local Nusa Penida temperature regimes during much of the year. Overall, temperature appeared an unlikely main driver of the sunfish season and instead, we suggest a presumed increase in sunfish prey availability, associated with the seasonal cold-water upwelling, probably causes an ‘influx’ of sunfish to the Lombok Strait, including to the reefs where they are seen by tourist divers.

4.2 Introduction

Human fascination with marine megafauna allows local communities around the world to benefit from nature-based tourism (e.g. O'Malley et al. 2013; Ward-Paige et al. 2013; Venables et al. 2016), however, if left unregulated, such industries may have detrimental impacts on the animals they target (e.g. Bejder et al. 2006; Semeniuk and Rothley 2008; Higham et al. 2016). One example is the iconic ocean sunfish, which is a popular tourism drawcard for Bali, Indonesia (Figure 4.1), where sunfish seasonally seek out cleaner-fish interactions on reef slopes in water depths accessible to recreational scuba divers (e.g. < 40 m) (Konow et al. 2006). A bustling SCUBA tourism industry has developed over the past ~three decades, in part driven by the rare opportunity to observe these enigmatic fish first hand. The most popular sunfish dive sites are found within the Nusa Penida Marine Protected Area (MPA) between the islands of Bali and Lombok (Figure 4.1). During the tourist high season, diver crowding and harassment of sunfish is a common occurrence (S Faust, All4Diving; A Taylor Blue Corner Dive; S Bebe, World Diving, pers comm 2018), interrupting sunfish – cleaner fish interactions, with unknown long-term biological and ecological consequences. Diver disturbances to the sunfish are currently addressed through a voluntary Diver Code of Conduct, implemented through the Nusa Penida MPA framework (Pokja KKP NP 2012), however, adherence to the code varies considerably between dive operators.

Sunfish sightings are highly seasonal, and are anecdotally highest during July – September/October (Welly et al. 2011; Pokja KKP NP 2012; Thys et al. 2016), approximately coinciding with the tourist high season (August – September; Ruchimat et al. 2013; Berdej and Armitage 2016). This 'sunfish season', is anecdotally linked to a decrease in sea surface temperature during the southeast monsoon, however it has been suggested that sunfish are present in the area year round, but inhabit deeper, cooler waters beyond recreational SCUBA diver depth limits, avoiding warm surface waters during the northwest monsoon (Thys et al. 2016).

The Bali sunfish are locally known as 'mola-mola' and are typically referred to as *Mola mola* in the literature (Konow et al. 2006; Ruchimat et al. 2013; Berdej and Armitage 2016) (but see Thys et al. 2016), however, they correspond morphologically to the world's heaviest bony fish *M. alexandrini* (Ranzani 1839) *sensu* Sawai et al. (2018), formerly *M. ramsayi* (Giglioli 1883). As for all Molidae species, limited life history information is available, although several satellite tagging studies in recent years have greatly increased the understanding of sunfish diving behaviour and habitat use (e.g. Potter and Howell 2010; Thys et al. 2015; Sousa et al. 2016b,c). These studies have predominantly targeted *M. mola*, and have shown that deep foraging dives up to several hundred meters are interspersed with sea surface basking, or time spent in the mixed layer above the thermocline, for thermoregulation purposes (e.g. Potter and Howell 2010; Dewar et al. 2010; Nakamura et al. 2015). Data is scarce for *M. alexandrini*, with limited satellite tagging of this species to date (Thys et al. 2016, 2017).

The conservation status of *M. alexandrini* has not been assessed separately from *M. mola*, which was listed as vulnerable by the International Union for Conservation of Nature in 2015 (Liu et al. 2015). There is scarce information on global sunfish population numbers, and while they may be a relatively numerous group (Breen et al. 2017; Grémillet et al. 2017), there is little clarity on the anthropogenic pressures on individual species (e.g. Nyegaard et al. 2018a) [Chapter 3]. Currently, the Indonesian government body, Ministry of Marine Affairs and Fisheries has placed sunfish on their work plan with a view for national protection, in part due to concerns of tourism related impacts in the Bali area (M Welly pers com 2018). However, there is at present little information to inform an assessment of the need for regulation, as many basic aspects of the seasonal Bali sunfish phenomenon are unknown, including what drives the intriguing seasonality and if more than one species frequents the Bali and Nusa Penida reefs.

The objectives of this study were to verify the species identity/identities of the Bali sunfish; establish the timing of the 2015 sunfish season in relation to the diver tourism high season, temperature regimes and major oceanographic features of the region; investigate the sunfish affinity to the NP MPA during the sunfish season; and explore the sunfish water column usage and dive behaviour to establish activities and temperature preferences. We achieved this by verifying the species genetically (mtDNA D-loop) and morphologically. We used environmental data, diver observations and satellite telemetry to gain an understanding of the seasonality and movement patterns of *M. alexandrini* in the Bali area, and assess if temperature is the likely driver of the seasonality. Based on the findings, we comment on the likely vulnerability of the Bali sunfish to diver crowding.

4.3 Material and methods

4.3.1 Introduction to the study area

The Nusa Penida Marine Protected Area (NP MPA) encompasses the three small islands of Nusa Penida, Nusa Lembongan and Ceningan, near the popular tourist destination of Bali (Figure 4.1). The climate in the region is predominantly influenced by the Asian-Australian monsoon system, characterized by six-month reversals in the winds associated with the southeast and northwest monsoons, linked to the intra-annual positions of the Inter Tropical

Convergence Zone (Gordon 2005; Susanto et al. 2007). The southeast and northwest monsoons systems are generally prevalent during the months of April – October (peaks in June – August), and October – April (peaks in December – February), respectively (Susanto and Marra 2005; Susanto et al. 2007).

The NP islands are located in the southern Lombok Strait between the islands of Bali and Lombok. The Lombok Strait comprises a deep channel (> 1,000 m depth), rising sharply to rugged sill areas of ~200 m depth between Nusa Penida and Lombok, and between Nusa Penida and Bali (Badung Strait) (Mitnik et al. 2000; Hendrawan et al. 2011; Mayer and Damm 2012) (Figure 4.1). To the south, the depths plummet rapidly to several thousand meters.

The Lombok Strait constitutes one of three passage ways in the lower Indonesian Archipelago for the Indonesian Through Flow, which brings surface waters from the Pacific Ocean into the Indian Ocean, via the Makassar Strait (Gordon 2005; Tillinger 2011; Sprintall and Révelard 2014) (Figure 4.1). During the southeast monsoon, the Indonesian Through Flow is generally a well-defined southward flow through the Lombok Strait, while the flow is absent, intermittent or reversed during the northwest monsoon and transitional periods (Susanto et al. 2007; Sprintall et al. 2009; Matthews et al. 2011). During the southeast monsoon, the Lombok Strait is also influenced by seasonal Ekman-driven upwelling of cold, nutrient-rich water along the southern border of Bali and neighbouring islands, which triggers a seasonal increase in primary productivity (Hendiarti et al. 2004; Gordon 2005; Susanto and Marra 2005). The oceanography inside the Lombok Strait is highly complex, influenced by strong, north-south bi-directional tidal currents (up to ± 3.5 m/s), internal waves (observed wavelengths of ~2 – 7.25 km and amplitudes exceeding 100 m), and dramatic short-term (min, h) up- and down welling events across the Lombok Strait sill areas (Mitnik et al. 2000; Susanto et al. 2005; Aiki et al. 2011; Hendrawan et al. 2011), causing extensive vertical mixing and turbulence of the water column. The thermocline depths (22°C isotherm; Susanto et al. 2000) to the north and south of the Lombok Strait are relatively stable, with long-term means of 106 and 94 m, respectively (Susanto et al. 2007). In comparison, inside the Lombok Strait the isohaline and isothermal layer is ~30 – 50 m (Mitnik et al. 2000), with highly volatile water column structures in the Lombok Strait sill areas, influenced extensively by tidally driven up and down-welling events (Hendrawan et al. 2011)

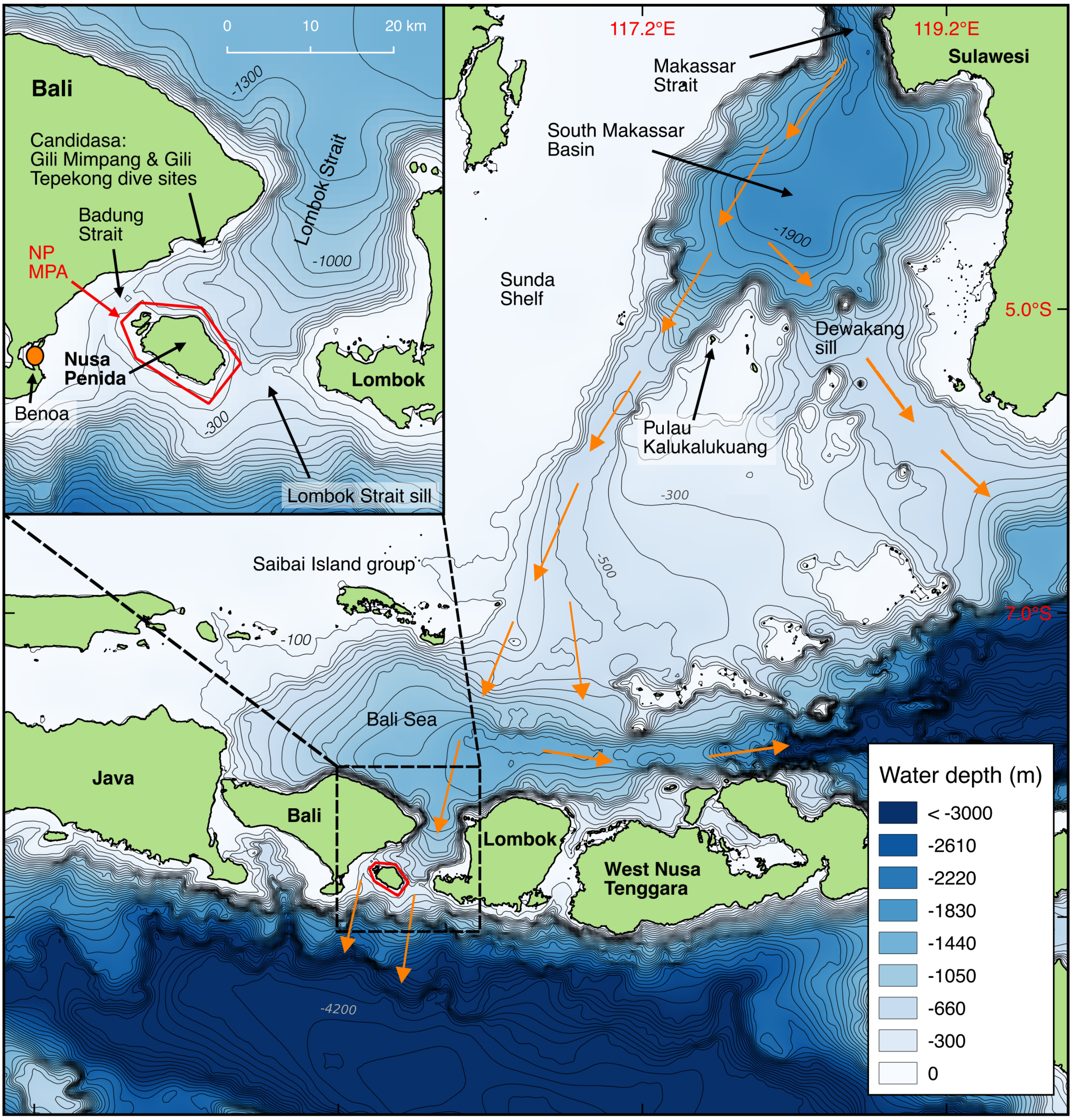


Figure 4.1 Bathymetry of the Lombok Strait and surrounding area. Red polygon is the Nusa Penida Marine Protected Area (NP MPA), solid orange arrows indicate the main flow path of the Indonesian Through Flow (Mayer & Damm 2012). Bathymetry from Gebco (*http://www.gebco.net*; GEBCO_2014 Grid, version 20150318).

4.3.2 *Water temperature*

Mean monthly satellite sea surface temperature (SST) (Aqua MODIS, 11 µ night-time, 4 km grid) composites for 2015 for the Lombok Strait were downloaded from National Oceanographic and Atmospheric (NOAA) [https://oceancolor.gsfc.nasa.gov]. Hobo Pendant and Pro v2 temperature loggers were deployed throughout 2015 at 10 and 30 m at four dive sites within the Nusa Penida MPA, namely at 'Sental' and 'Manta Bay' on the Nusa Penida north and southwest coast, respectively, and at 'Toyapakeh' and 'Crystal Bay' in the north and south part of the Nusa Penida channel, respectively (Figure 4.2). In all instances, the loggers recorded ambient water temperature in 10 min intervals. Complete, or near-complete time series were obtained from Sental (10 m), Toyapakeh (10 and 30 m) and Manta Bay (30 m), while logger loss or malfunction resulted in partial data loss at Crystal Bay (10 and 30 m), Manta Bay (10 m) and Sental (30 m).

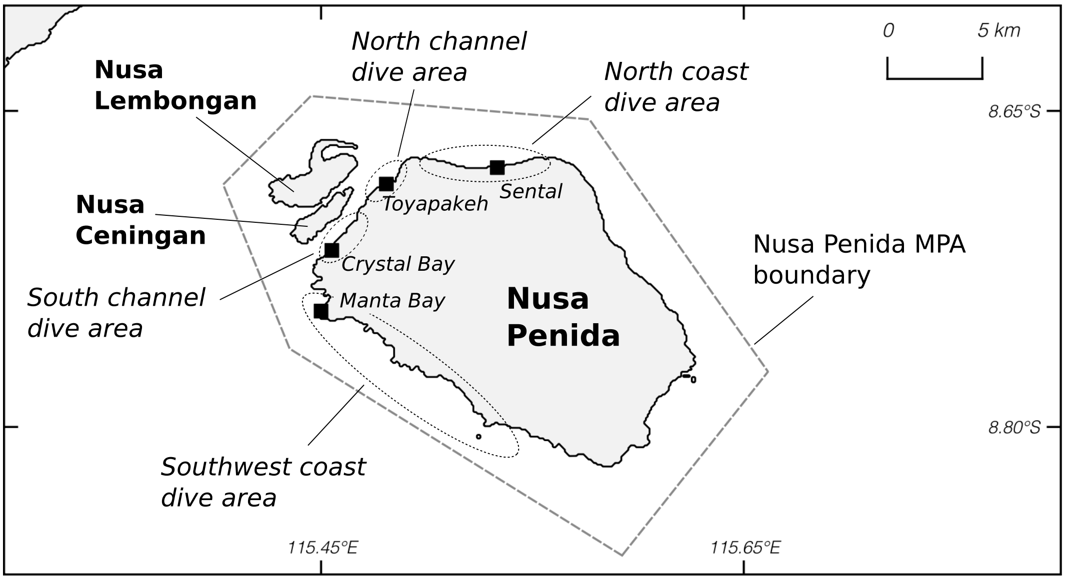


Figure 4.2 Temperature loggers deployed at four Nusa Penida dive sites (black boxes). The North- and Southwest coast dive areas encompass all diving on the North and Southwest Coast, respectively (individual dive sites are not clearly delineated in these areas); North channel dive area encompasses dives in the general Toyapakeh area; South channel encompasses Crystal Bay and surrounding area. The Nusa Penida Channel is situated between Nusa Penida and Ceningan/Nusa Lembongan.

4.3.3 *Sunfish season*

The timing of the sunfish season was established by examining tourist dive operator data from a small subset of dive operators from the area. All dives undertaken between January – December 2015 in the Nusa Penida MPA by seven dive operators participating in this study, based either on Lembongan (*n* = 3) or Bali (*n* = 4), were recorded. In addition, dives at the popular sunfish sites Gili Mimpang and Gili Tepekong off Candidasa, Bali (Figure 4.1) were recorded. One set of dive data was collected from each boat (dive site, date, dive start time, maximum dive depth, number and depth of sunfish seen), following the dive guide with the deepest maximum depth at each site. The data were collected daily or weekly by the first author (MN) with help from volunteers, and we gauge that > 95% of all dives undertaken by the participating dive operators were captured.

In total, 5,440 Nusa Penida MPA dives were recorded. Approximately 80% of these were undertaken at a small number of popular dive sites where sunfish are regularly seen, and these were grouped into four 'dive areas' (Figure 4.2) based on dive site proximity, and a subjective judgment of similarity of underwater topography, current conditions, substrate and sunfish encounter rates (M Nyegaard pers obs). The remaining dives (< 20%) were not easily grouped, or were from sites where sunfish are rarely seen, and were removed from further analysis. The resulting data set consisted of 4,427 dives, during which a total of 707 individual sunfish were sighted. In addition, 165 dives were recorded from Gili Mimpang and Gili Tepekong off Bali (Figure 4.1) ('Candidasa dive area'), with a total of 48 sunfish encounters. Monthly sunfish encounter rates (sunfish.dive^{-1}) were calculated for each dive area separately. In addition, 74 dedicated sunfish research dives were undertaken within the Nusa Penida MPA between 12 August and 11 October 2015, away from tourist activities. During these dives, 115 sunfish were encountered, and as these dives were targeted for sunfish and had highly comparable dive profiles, a higher resolution (fortnightly) encounter rate estimations were warranted.

4.3.4 Satellite tagging

Between 21 August and 21 September 2015, eight sunfish (Table 4.1) were tagged within the Nusa Penida MPA with towed Argos-linked Fastloc GPS tags [Wildlife Computers (WC) SPLASH10-F-321A]. We used titanium darts and 100 cm stainless steel tethers (50 cm for Fish 1; Table 4.1). The tags were attached near the posterior edge of the dorsal fin by a SCUBA diver, using a 65 cm pneumatic spear gun fitted with a WC dart applicator and a 'stopper' to prevent the dart penetrating beyond 10 cm. All tags were programmed to detach after six months by way of corrosion of a small tether pin using the tags battery power. The total length of each fish was estimated from footage post-dive, by comparing the relative length of the tether (with known lengths) against the relative length of the fish.

Depth and temperature was recorded by the tags every second, and summarised into 50 m depth intervals between 0 – 400 m (as well as 0 – 1 m, 1 – 10 m, 400 – 600 m and >600 m), and into 2°C temperature bins between 5 and 29°C. Two different histogram periods were used: 12 hr (6 am – 6 pm; 6 pm – 6 am; $n = 4$ tags) to investigate day-night differences in water column occupancy, and 24 hr (6 am – 6 am; $n = 4$ tags) to reduce the number of Argos transmissions (Table 4.1). In addition, hourly SST (< 10 m) and maximum depths were collated by the tag. Depth and temperature time series were recorded in 7.5 min intervals (5 min for Fish 1) for the first three days after tagging, then for two of every seven days subsequently (three of every 16 days for Fish 1), along with minimum and maximum 6-h values.

The tags recorded three types of location data: Global Positioning System (GPS) Fastloc™ positions during very short tag surfacing events (accuracy of 10s – 100s m; Hazel 2009; Dujon et al. 2014); Argos locations when tag surfacing events coincided with polar orbiting CLS Argos satellite overpasses (accuracy of 10s – 100s km; Argos 2018); and light level data in 10 second intervals, processed into light intensity curves by the on-board tag processer for later modelling of light-based geolocations (accuracy of 100s km; Lam et al. 2010; Basson et al. 2016). To avoid battery drainage, the tags were restricted to a daily maximum of two successful GPS location acquisitions, and 15 attempts (8 in any one hour). After tag release (all tags detached prematurely), processed data were transmitted through Argos and decoded through the WC online Data Portal. GPS Fastloc information was post-processed by the WC GPS solver to yield GPS positions.

4.3.4.1 Light-based geolocations

Light-based geolocations were modelled through the WC online Global Position Estimator (GPE3) statistical processing tool, which uses a diffusion based movement model to estimate most-likely fish positions from light intensity curves, seed-locations (tagging position, Argos and Fastloc GPS positions), and from SST and depth readings cross-referenced with satellite SST (NOAA OI SST V2) and bathymetry (ETOP01-Bedrock bathymetry; Amante & Eakins, 2009). The model generated gridded (0.25° latitude/longitude) 12 h probability surfaces, and derived the most-likely fish positions at the time of each observation as well as twice-daily at midday and midnight. The 12 h probability surfaces were averaged across the period at liberty for each fish to produce average residency distributions.

While *M. mola* and *M. alexandrini* typically move at long-term horizontal speeds of < 1 m/s (e.g. Sims et al. 2009a; Thys et al. 2015, 2017), the GPE3 was run with three different animal speeds (1, 2 and 3 m/s), to test model performance. We also ran the GPE3 with and without

SST data, as well as without GPS Fastloc locations, providing the model with tagging and detachment positions only, emulating traditional pop-up archival tags.

4.3.4.2 Statistics

To investigate if larger fish tended to dive deeper, estimated total fish length was tested against maximum recorded dive depth, as well as mean and median time spent > 300 m, using the non-parametric Spearman Rank Correlation test, as a linear relationship between depth and size cannot be assumed. To compare depth occupancy and exposure to various temperature ranges between fish we used the non-parametric Man-Whitney U-test, as all data sets failed the Shapiro-Wilks W tests for normality (results not shown).

4.3.5 Specimen identification

On three occasions, skin residue remained on the dart applicator after tagging (Table 4.1). These samples were secured under water, and transferred to a vial with RNALater post-dive. DNA extraction, PCR of the mtDNA D-loop, Sanger sequencing and phylogenetic analysis followed (Nyegaard et al. 2018a) [Chapter 3], using a subset of published sequences as references. All tagged fish were filmed, and the species identity verified post-dive according to (Sawai et al. 2018). The Bali Sunfish ID Catalogue (*http://www.thebalisunfish.org*), a large, unpublished collection of verified tourist photographs of sunfish from the Bali area, was reviewed and species identities determined morphologically.

4.4 Results

4.4.1 Water temperatures

The monthly satellite SST composites (Figure 4.3) revealed two main temperature regimes in the Lombok Strait during 2015; warm water capping the Lombok Strait and surrounding area (January –April; December), and cooler surface waters entering the Lombok Strait from the north concurrent with cold water upwelling along the southern coastline of Bali and neighbouring islands (July – October). Transitions between these two regimes occurred during May/June and November 2015 (Figure 4.3).

At the four sites where loggers were deployed, the temperature data from both 10 and 30 m revealed extensive short-term oscillations (min, h) (Figure 4.4), more so at 30 m than 10 m (e.g. Figure 4.5). The temperature oscillations were irregular within and between sites, but were, as expected, related to the tidal cycles (e.g. Figure 4.4, Figure 4.5). The temperature at Sental on the Nusa Penida north coast was similar between 10 and 30 m, and oscillated much less than all other sites (10 m data shown; Figure 4.5A). The overall oscillation patters throughout the year at all sites and both depths (although less so at Sental) appeared to be related to the moon phase, with the most intense cold-water events generally occurring just after full and new moon (Figure 4.4).

The loggers on the north and southwest coast (Sental and Manta bay, respectively) were predominantly influenced by the Indonesian Through Flow (from the north) and seasonal cold water upwelling (from the south), respectively (e.g. black and white arrows on 'July' in Figure 4.3). Overall, the two loggers substantiated the timing of the increased intensity of the Indonesian Through Flow and the seasonal cold-water upwelling, as determined from SST observations (Figure 4.3). Specifically, a decreasing temperature trend started on the north coast in ~July, lasting until the end of October, with overall lowest temperatures in September (Figure 4.4A,B). On the southwest coast the temperature started to drop earlier, (May/June), lasting into November (Figure 4.4G.H). Particularly low temperatures occurred during mid-August – mid-October, with the coldest temperature regime in September.

The seasonal temperature trends in the north and south of the Nusa Penida Channel (Toyapakeh and Crystal Bay, respectively) approximately followed the north and southwest coast temperature regimes, with warmer temperature regimes in the north channel. However, both channel sites were highly influenced by small-scale temperature oscillations during most of the year, so much so that the overall coldest months occurred during October at both sites.

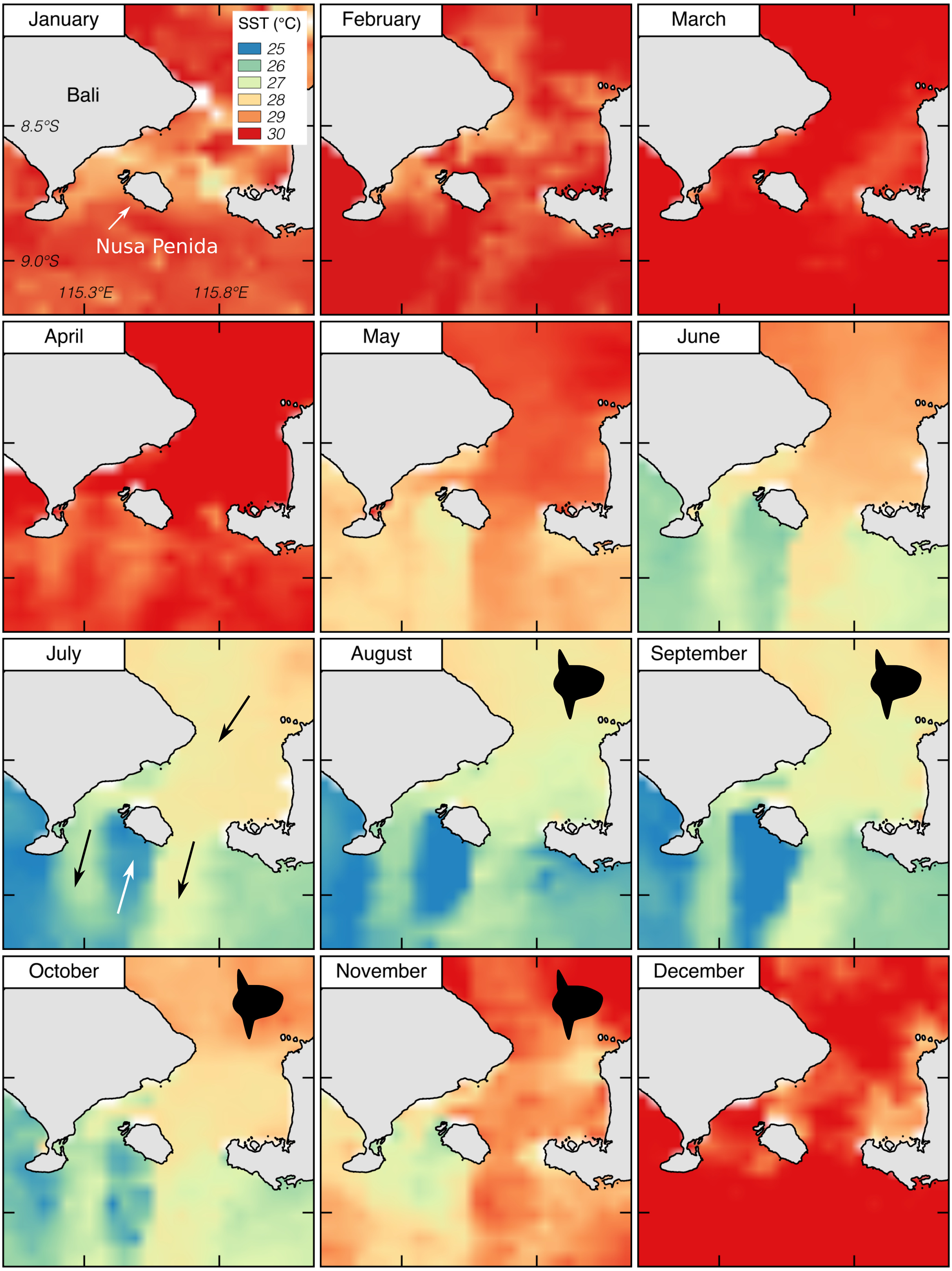


Figure 4.3 Average monthly Sea Surface Temperature in the Lombok Strait During April – December 2015, interpolated from Modis Aqua OceanColor 4 km (11 µ night-time) monthly composites [National Oceanographic and Atmospheric (NOAA): *https://oceancolor.gsfc.nasa.gov*]. Sunfish season (black sunfish symbols) as determined by dive operator sightings data (see text for details). Black arrows (July) indicate Indonesian Through Flow, white arrow indicates upwelling.

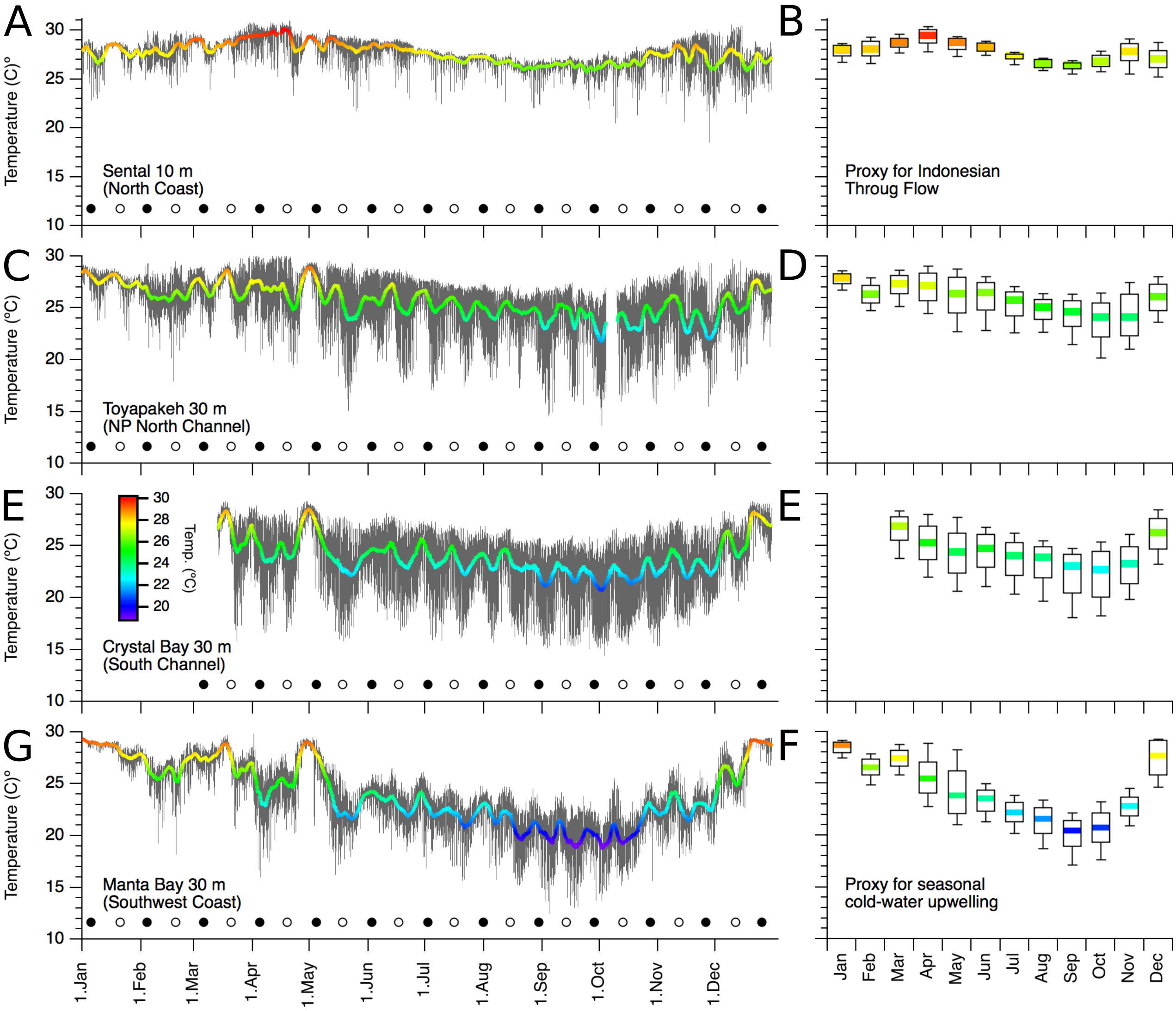


Figure 4.4 Temperature in 10 m intervals at **A,B)** Sental (10 m) on the Nusa Penida North Coast, **C,D)** Toyapakeh (30 m) in the North Channel, **E,F)** Crystal Bay (30 m) in the South Channel, and **G,H)** in Manta Bay (30 m) on the Southwest Coast. Grey lines on left panels are raw temperature data, with the 3-day rolling mean superimposed (colour-coded line). Black and white circles are full and new moon, respectively. Right panels show box plots of the same data. Middle (colour-coded) box lines are monthly medians, lower and upper box edges are 25 and 75 percentiles, respectively, and whiskers are 10 and 90 percentiles, respectively.

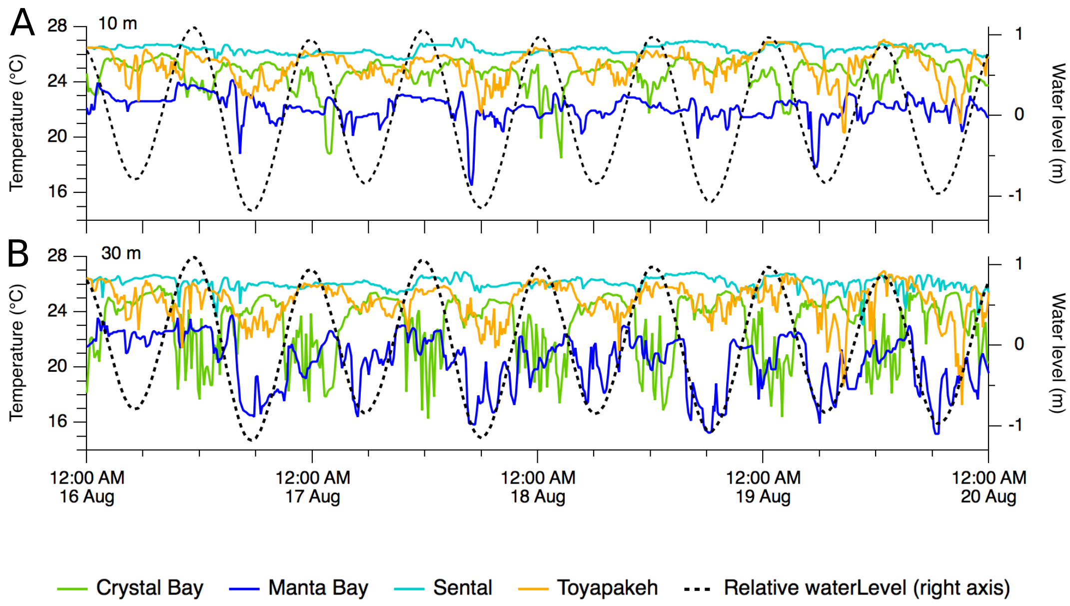


Figure 4.5 Temperature in 10 min intervals at **A)** 10 m, and **B)** 30 m depth on the four temperature logger sites in the Nusa Penida Marine Protected Area (Figure 4.2). Tide data from Benoa station (Figure 4.1), obtained from the University of Hawaii Sea Level Center (https://uhslc.soest.hawaii.edu/data/) (Caldwell et al., 2015).

4.4.2 Dive tourist and sunfish seasons

The total number of monthly dives in the Nusa Penida MPA by the three participating Nusa Lembongan operators, representing all their dive activity for the year, varied seasonally (Figure 4.6A). Combined, the monthly number of dives were similar during January – June (average ± 1 SE = 302 ± 14), followed by a sharp increase during July – September (543 ± 16), with a peak of 554 – 563 in July/August, and a decrease from October to December (293 ± 55).

The maximum depths of all Nusa Penida MPA dives from the participating dive centers (n = 7), versus dives where sunfish were seen, varied between 2 – 47 m and 12 – 47 m, respectively (Figure 4.6B). The depths of encountered sunfish were estimated on 625 occasions, and varied between 1 – 50 m. To reduce bias, dives with maximum depths ≤ 12 m were removed from the data set prior to calculating mean monthly sunfish encounter rates (sunfish.dive^{-1}).

The peak mean monthly encounter rates varied between the five dive areas (Figure 4.7). The highest monthly rates occurred in the South Channel during September (peak rate ± 1 SE = 1.39 ± 0.13 sunfish dive-1) and at Candidasa off Bali in October (1.0 ± 0.36). The peak rates were substantially lower in the North Channel (October: 0.31 ± 0.11), on the North Coast (September: 0.21 ± 0.05), and Southwest Coast (September: 0.18 ± 0.04). Despite these differences, a similar seasonal pattern was evident in four of the five areas (Figure 4.7B,E), with no or low sunfish encounter rates between January and July/August, increased rates from August/September to October/November, followed by a decrease in November/December. While the fortnightly research dive encounter rates were substantially higher than for tourists (Figure 4.7F), they corroborated the tourist encounter rate pattern, with gradually increasing rates during August and the first half of September, a distinct peak during the second half of September (3.71 ± 0.99) followed by a decrease in the first part of October. The seasonal encounter rates on the North Coast (Figure 4.7A) differed from all other areas with slightly elevated encounter rates during most of the year, and with a second peak in April in addition to September.

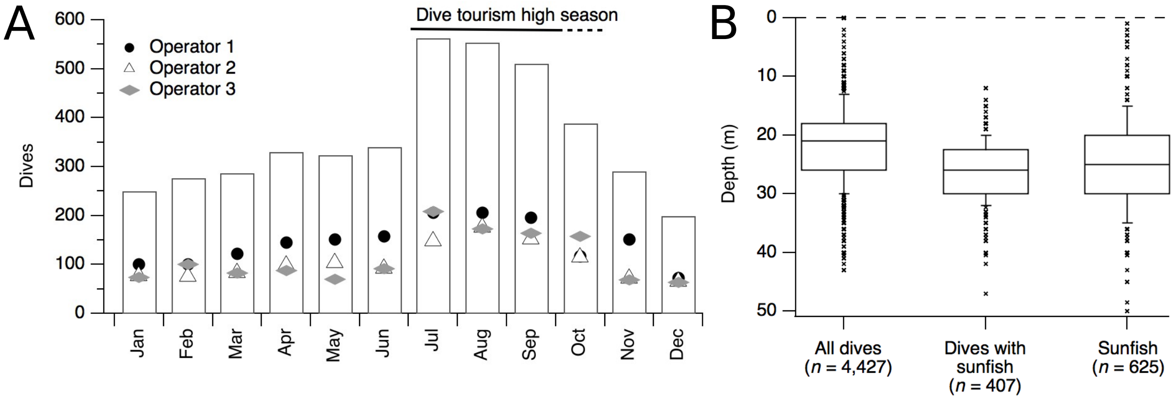


Figure 4.6 **A)** All dives recorded by the three participating Nusa Lembongan dive operators during 2015, and **B)** maximum depths of dives within the Nusa Penida Marine Protected Area recorded by all seven participating dive operators (Nusa Lemongan operators *n* = 3, Bali dive operators *n* = 4) during 2015. The depths of encountered sunfish were estimated on 625 occasions and varied between 1 – 50 m. Middle box lines are monthly medians, lower and upper box edges are 25 and 75 percentiles, respectively, and whiskers are 10 and 90 percentiles, respectively, crosses represent data points beyond 10 and 90 percentiles.

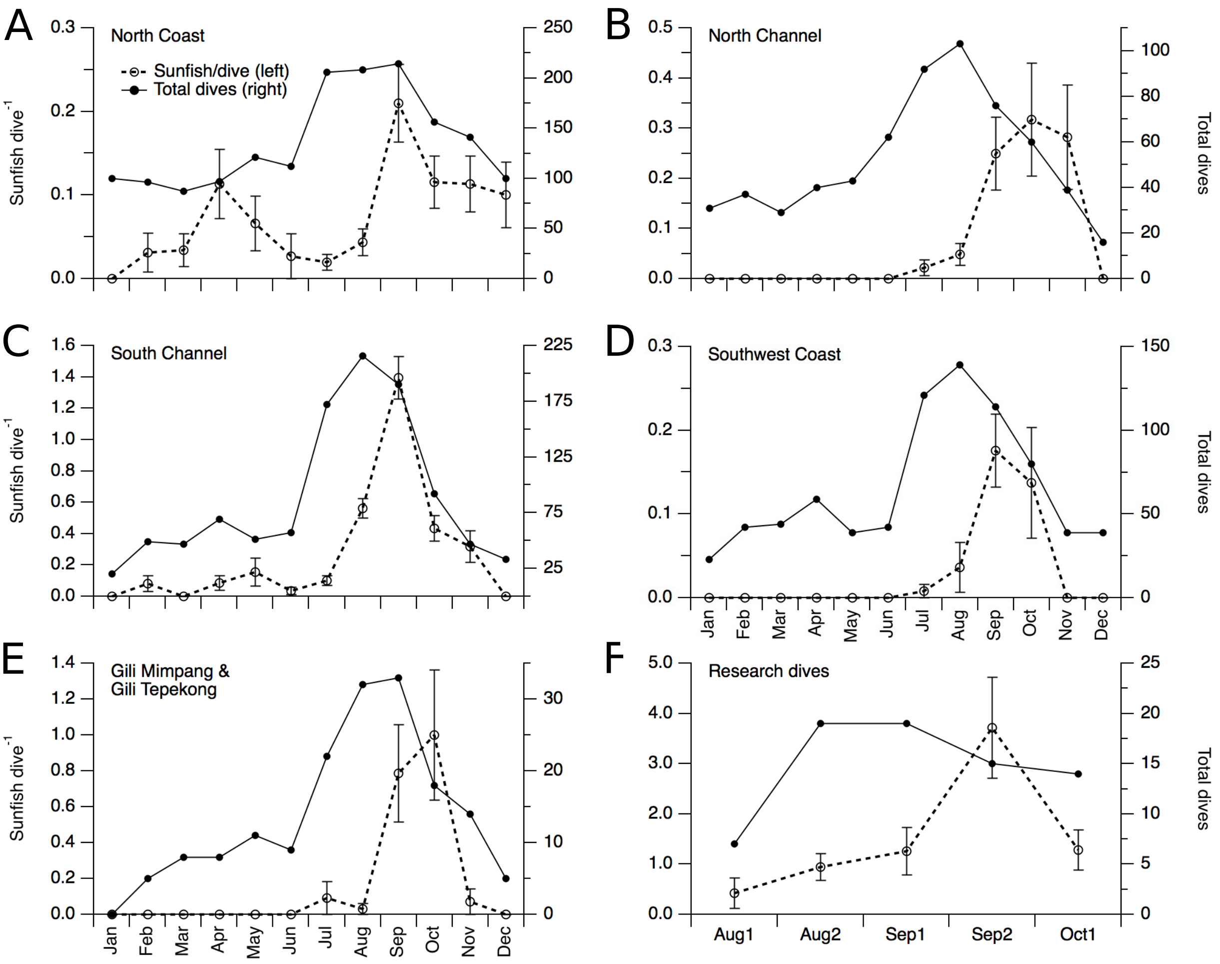


Figure 4.7 Average monthly sunfish.dive^{-1} (open circles, broken lines; left axis) based on total dives recorded from all dive operators (closed circles, black lines; right axis) in the dive areas **A)** North coast, **B)** North channel, **C)** South channel, **D)** Southwest coast(Figure 4.2), and **E)** Gili Mimpang and Gili Tepekong (GMGT) on Bali (Figure 4.1), and **F)** during targeted sunfish research dives within the Nusa Penida Marine Protected Area. Error bars are 1 Standard Error of the mean.

4.4.3 Specimen identification

The eight tagged fish were estimated at 1.4 – 1.6 m total length (TL) (Table 4.1). All fish had similar morphologies, corresponding to *M. alexandrini* (Sawai et al. 2018), each with developing head and chin bumps, and a rounded clavus. All three mtDNA D-loop sequences nested in the *Mola* sp. A genetic clade of (Yoshita et al. 2009), corresponding to *M. alexandrini* (Sawai et al. 2018) (results not shown). Furthermore, all three sequences nested in the proposed 'subtropical' clade of *M. alexandrini* (Yoshita et al. 2009; Nyegaard et al. 2018a [Chapter 3]). The sequences were uploaded to GenBank under Accession numbers MH104868 – MH104870. A total of 757 verified image submissions from the Bali Sunfish ID Catalogue, captured between 2000 and 2017, both during and outside the sunfish season, were of sufficient quality to confidently assess the species identity, with all but one individual identified as *M. alexandrini*. A single *Ma. lanceolatus* was filmed in July 2017 off Toyapakeh, i.e. in the Nusa Penida Channel (Figure 4.2).

4.4.4 Horizontal movements

All eight satellite tags detached prematurely from the fish with the corrosion pin still intact. Six tags stayed attached to the sunfish between 17 – 76 days, while two tags detached within 36 h (Table 4.1). After detachment, six tags transmitted between 6.8 – 49.4, while two stopped transmitting almost immediately. The combination of early detachment and transmission failures resulted in two tags yielding partial, but usable data sets (Fish 3 – 4), while three tags yielded very little data (Fish 5 – 7) (Table 4.1).

A total of 105 GPS Fastloc positions were captured and transmitted via Argos by the eight tags combined. Each GPS Fastloc positions was based on computations of 4 – 8 satellites, yielding position accuracies of tens of meters (8 satellites) to hundreds of meters (4 satellites) (Hazel 2009; Dujon et al. 2014). Very few Argos positions (total of 16) were obtained during the deployment periods, and most of these (78%) had no accuracy estimation (class A or B; Argos, 2018) (Table 4.1).

During deployment, the five most successful tags (100 cm tether) captured on average 0.3 – 0.8 GPS Fastloc locations.day-1, whereas the tag on Fish 1 (50 cm tether) had the lowest acquisition rate of 0.1 Fastloc.day^{-1} (Table 4.1). Only two GPS locations (including pop-up location) were recovered from Fish 6, but this may have been an underestimate due to transmission failure almost immediately after tag detachment.

Two different GPS Fastloc position patterns were evident between the eight tagged fish. The tags on six fish mainly captured GPS positions in the Lombok and Badung Strait sill areas, in close proximity to Nusa Penida, and along the Bali coastline in the western Lombok Strait (Figure 4.8A). These results included two fish, which both travelled from Nusa Penida after tagging, across the Badung Strait to the Candidasa dive area off Bali, where the tags on both fish detached prematurely within 36 h. The second major movement pattern was exhibited by two fish, which both travelled out of the Lombok Strait. The tag on one fish detached after 17 days in the Bali sea, while the other continued north via the Saibai island group, towards the South Makassar Basin, where several GPS positions were obtained along the steep seabed gradient west of Pulau Kalukalukuang (Figure 4.8B).

The GPE3 model output overall corresponded with the wider GPS Fastloc location patterns when modelled with fish speeds of 1 m/s (blue inserts on Figure 4.8), however despite an increased model score, the results became highly unrealistic when fish speed was increased (Appendix A3.2). In summary, the model relied heavily on the GPS positions to place the fish in or near the Lombok Strait, and including SST data did not notably alter the results (Appendix A3.2). Light-based geolocation accuracy is generally low, however the particular conditions in this study likely exacerbated the inaccuracy due to the proximity to the equator and the equinox, where day length vary little, combined with sunfish deep diving behaviour from dawn – dusk, introducing inaccuracies in determining the time of twilight (Lam et al. 2010).

4.4.5 Vertical movements

Acceptable Argos message recovery of Time-at-Depth (TAD) and Time-at-Temperature (TAT) data were achieved for five tags with > 36 h deployment periods (Table 4.1), and these originated from four fish, which stayed in the Lombok Strait (Fish 1 – 4; Figure 4.8A), and one fish, which travelled north (Fish 8; Figure 4.8B). Overall, 24-h mean TAD profiles were similar between the five fish, and revealed a wide use of the water column across all depth strata between 0 – 50 and to 400 – 600 m (Figure 4.9). However, the two most frequently occupied depth strata were slightly deeper for Fish 8, which swam north (50 – 150 m), than for Fish 1 – 4, which stayed in the Lombok Strait (0 – 100 m) (Figure 4.9A-E). All five fish spent little time at or near the surface, with mean occupancy at 0 – 1 m and 0 – 10 m ranging between 0.3 – 1.1% and 1.2 – 7.8%, respectively (Figure 4.9K,L). Time spent in the 400 – 600 m layer was likewise low for all fish (< 4 %). The max recorded depths were similar between Fish 1, Fish 2 and Fish 4 (464 – 472 m), and between Fish 3 and Fish 8 (552 m for both) (Table 4.1). The estimated total length was similar between the five fish (1.4 – 1.6 m), and neither maximum depth, nor mean or median daily TAD > 300 m were significantly related to the estimated sunfish length (R_s = 0.58, p = 0.30; R_s = 0.11, p = 0.87; R_s = -0.26, p = 0.67, respectively, *n* = 5 for all).

The five fish experienced a broad range of temperatures from 7 – 9°C to 27 – 29°C (Figure 4.9F – J), with overall similar mean TAT patterns between fish. Notably, however, Fish 8 (which travelled north) spent significantly more time in the warmest temperature range (27 – 29°C), despite spending significantly less time in the upper 50 m, compared with the four fish, which stayed in the Lombok Strait. Overall, the exposure to temperatures in the 27 – 29°C range was limited, in particular for Fish 1 – 4 (mean < 5% for all), although during a some 24-h periods this exposure ranged between 20 - 45% for Fish 1 and Fish 8 (Figure 4.9M).

4.4.5.1 Day-night differences in depth occupancy and temperature exposure

The tags on Fish 1 and Fish 8 were programmed to recover 12 h depth and temperature histograms, reflecting the day-night cycle in Bali, which vary little over the course of the year. Daytime TAD for both fish revealed variations within fish over time, however no discernible pattern was evident for Fish 1, which stayed in and around the Lombok Strait (Figure A3.2.4 in Appendix A3.2). Fish 8 also exhibited day-to-day differences, however, a major shift occurred on 9 October (Figure A3.2.4 in Appendix A3.2). Prior to this date (21 September – 8 October) the fish spent most of the daytime (78%) in < 150 m and a wide range of temperatures, after which it shifted its depth occupancy downwards, and spent most of the daytime (~75%) in depths > 200 m and in temperatures < 15°C (~80%) (9 – 31 October) (Figure 4.10B,C,E,F). Both daytime depth and temperature shifts were significant, and these differences were also significant when excluding data from the first 72 h of the deployment period, when Fish 8 was still in or near the Lombok Strait (Table 4.2).

During the night, both fish spent the majority of time in < 200 m (91 – 96%) (Figure 4.10A – C). However, the 0 – 50 m depth occupancy of Fish 8 was significantly lower during both periods compared with Fish 1 (Table 4.2). Despite of this, the exposure to 27 – 29°C was significantly higher for Fish 8 during both periods (Table 4.2).

4.4.6 Depth-temperature time series - water stratification

A total of 26 depth and temperature time series were recovered from the eight tags, primarily from Fish 1, Fish 2 and Fish 8 (Table 4.1). Combined, they revealed two types of water stratification, where the data points either fell on a relatively clean line and indicated stratified waters with a well-defined thermocline around 100 m (Figure 4.11A – D); or where data points were scattered with no discernible thermocline, or with a shallow thermocline < 50 m, indicating highly volatile thermal water column structures (hereafter referred to as ‘dynamic waters’) (Figure 4.11E – H). While both types of profiles were recorded throughout the tagging program, stratified waters were predominantly recorded by Fish 6 (*n* = 2) and Fish 8 (*n* = 5), which both travelled north out of the Lombok Strait (Figure 4.8B). Similar profiles were also recorded by Fish 3 (*n* = 1), shortly before the tag dislodged and popped up along the north coast of Bali (Figure 4.8A), as well as by Fish 1 (*n* = 1), but without any associated position information.

4.4.6.1 Depth-temperature time series – dive profiles

Overall, the recurrent 48-h depth and temperature time series revealed irregular dive patterns for all fish, both within and between 12-h daytime and night-time periods

(Figure 4.12). Generally, the fish dived deeper and to lower temperatures during the day, than during the night, shifting dive patterns around dawn and dusk. The daytime deep dives were generally long, at times several hours, with no or very short visits to 'shallower' waters (e.g. < 100 m) (Figure 4.12A – B). These dives were reminiscent of "U" type profiles (e.g. long periods at depth), considered to reflect foraging (Queiroz et al. 2017). Occasionally, short "V" type dives were also seen (e.g. very short duration at depth), considered to be "search dives" (*op. cit.*) (e.g. Figure 4.12B – D). Occasionally, daytime dive profiles revealed long periods (e.g. > 1 h) of consecutive depth recordings near the surface, in between deep dive activity (e.g. Figure 4.12C,D).

The profiles also revealed that fish occupying stratified waters (Figure 4.12E – H) appeared to spend less time near the surface then fish in 'dynamic waters' (Figure 4.12A – D), and instead spend time around the thermocline in between deeper dives, although they did not entirely avoid shallower waters < 50 m (e.g. Figure 4.12H).

Night-time dive patterns generally revealed less dive activity than during the day, however most nights included some dive activity to temperatures < 15°C. At times these were reminiscent of daytime dive profiles, although not as deep (e.g. Figure 4.12A – C). Conversely, some daytime dive patterns included little dive activity (e.g. Figure 4.12E,G,H), somewhat reminiscent of night-time dive patterns.

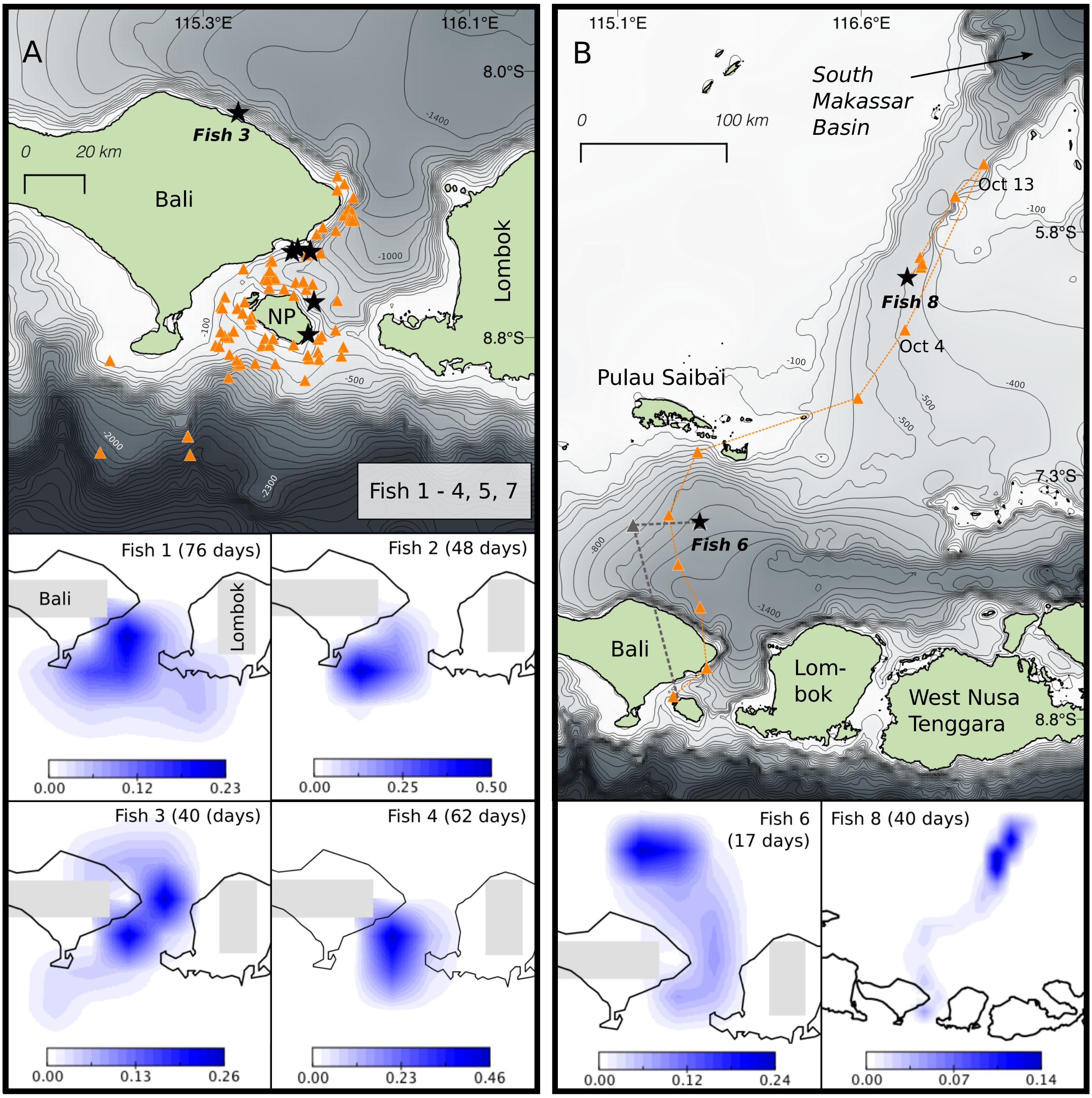


Figure 4.8 GPS Fastloc positions (orange triangles) and tag pop-up locations (black stars) for **A)** 6 fish between 21 Aug – 5 Nov 2015, with a combined total of 229 tracking days, including two fish with early tag detachment (Fish 5 after 14 h; Fish 7 after 39 h); and **B)** Fish 6 (13 September – 6 October 2015) and Fish 8 (21 September – 30 October 2015). Blue inserts are mean distribution across the period at liberty for each fish based on light-based geolocation modelling (GPE3) (grey squares in inserts represent land boundaries in the state-space model).

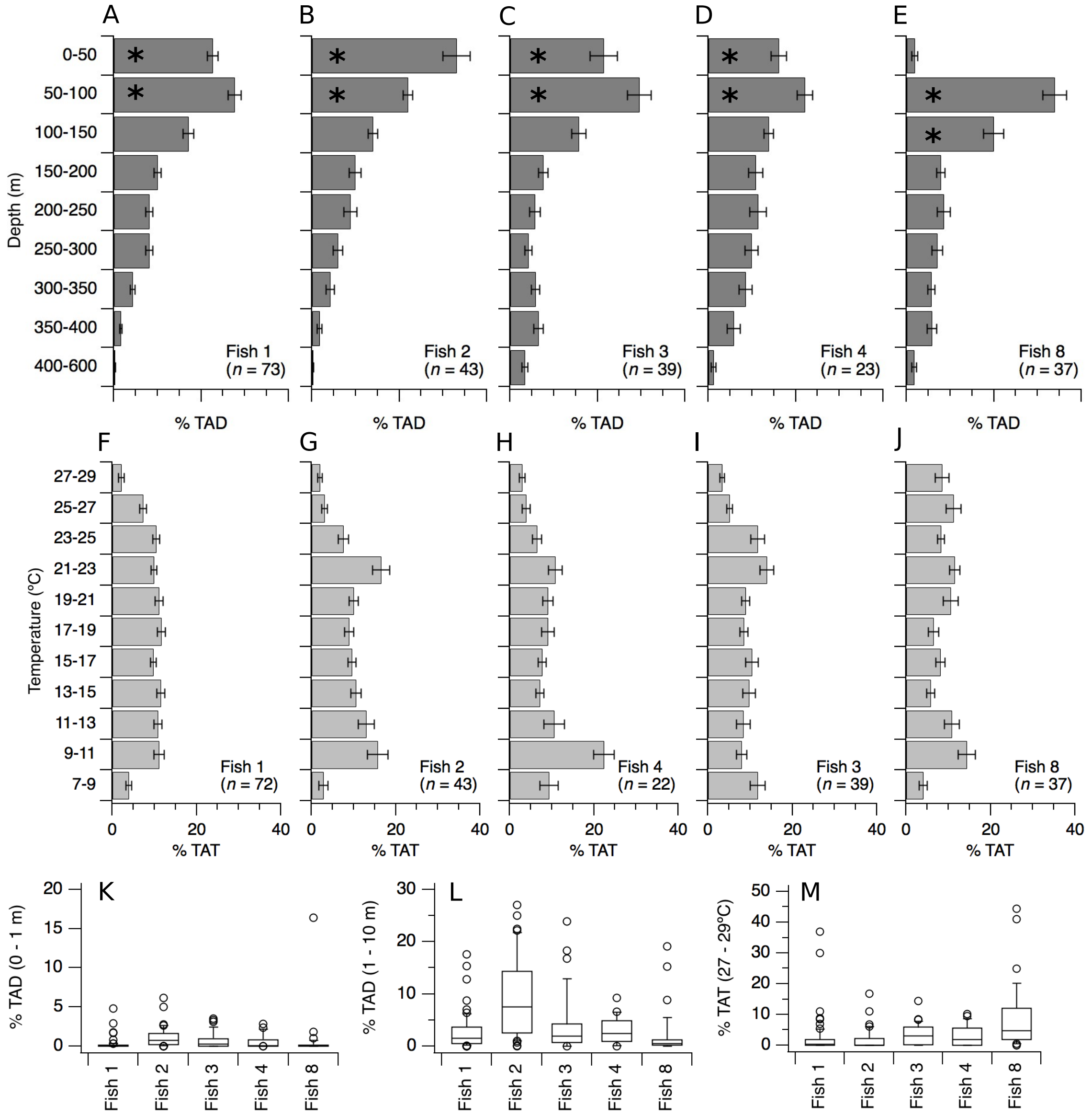


Figure 4.9 Mean 24-h Time at Depth (TAD) (upper panel) and Time at Temperature (TAT) (lower panel) for **A,F)** Fish 1; **B,G)** Fish 2; **C,H)** Fish 3; **D,I)** Fish 4, and **E,J)** Fish 8. Error bars are 1 standard error of the mean. *Denotes the two depth ranges with highest mean TAD for each fish. **K,L)** Box plots of TAD (0 -1 m) and TAD (1 – 10 m); and **M)** TAT (27 – 29°C) during 24-h periods (*n* for each fish as per upper and middle panels). Middle box lines are medians, lower and upper box edges are 25 and 75 percentiles, respectively, and whiskers are 10 and 90 percentiles, respectively, circles represent data points beyond 10 and 90 percentiles.

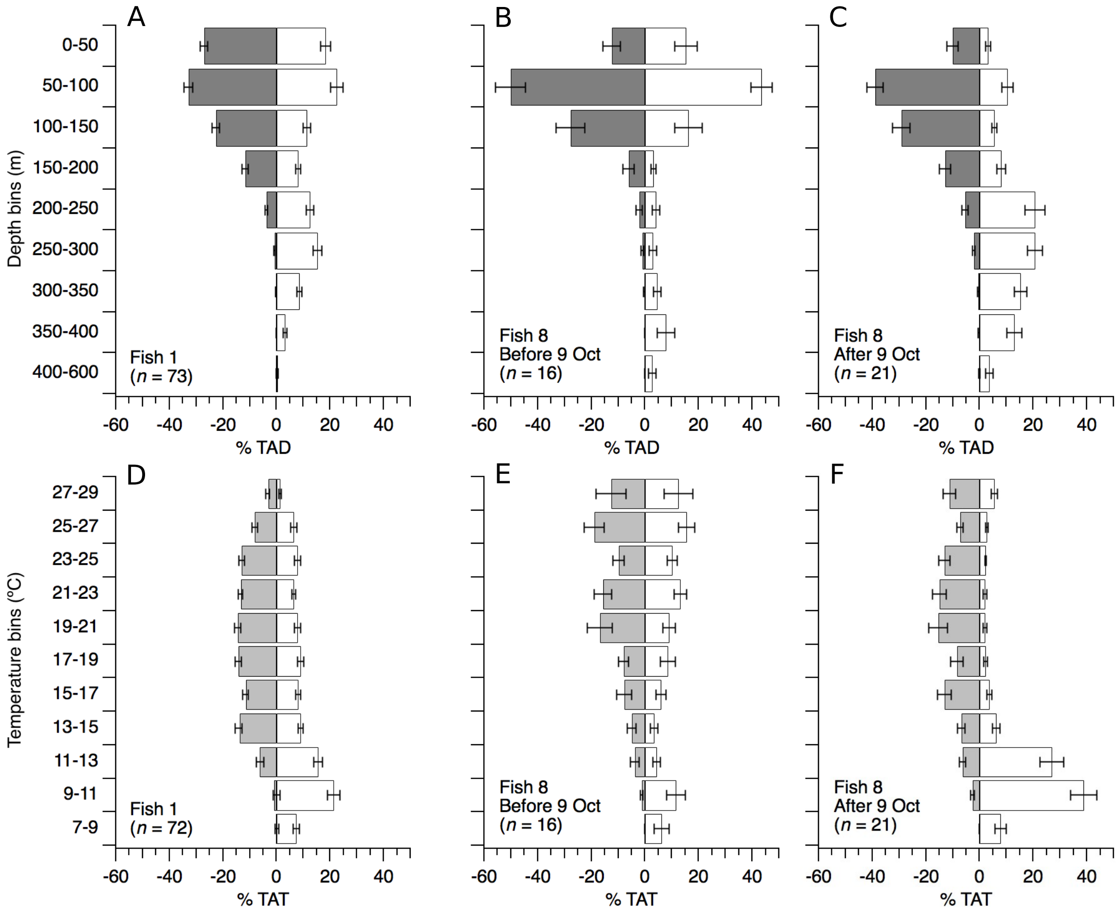


Figure 4.10 Mean 12-h Time at Depth (TAD) (upper panel) and Time at Temperature (TAT) (lower panel) during daytime (6 am – 6 pm; white bars) and night-time (6 pm – 6 am; grey bars) for **A,C)** Fish 1, **B,E)** Fish 8 before 9 October, and **C,F)** Fish 8 after 9 October. Error bars are 1 standard error of the mean.

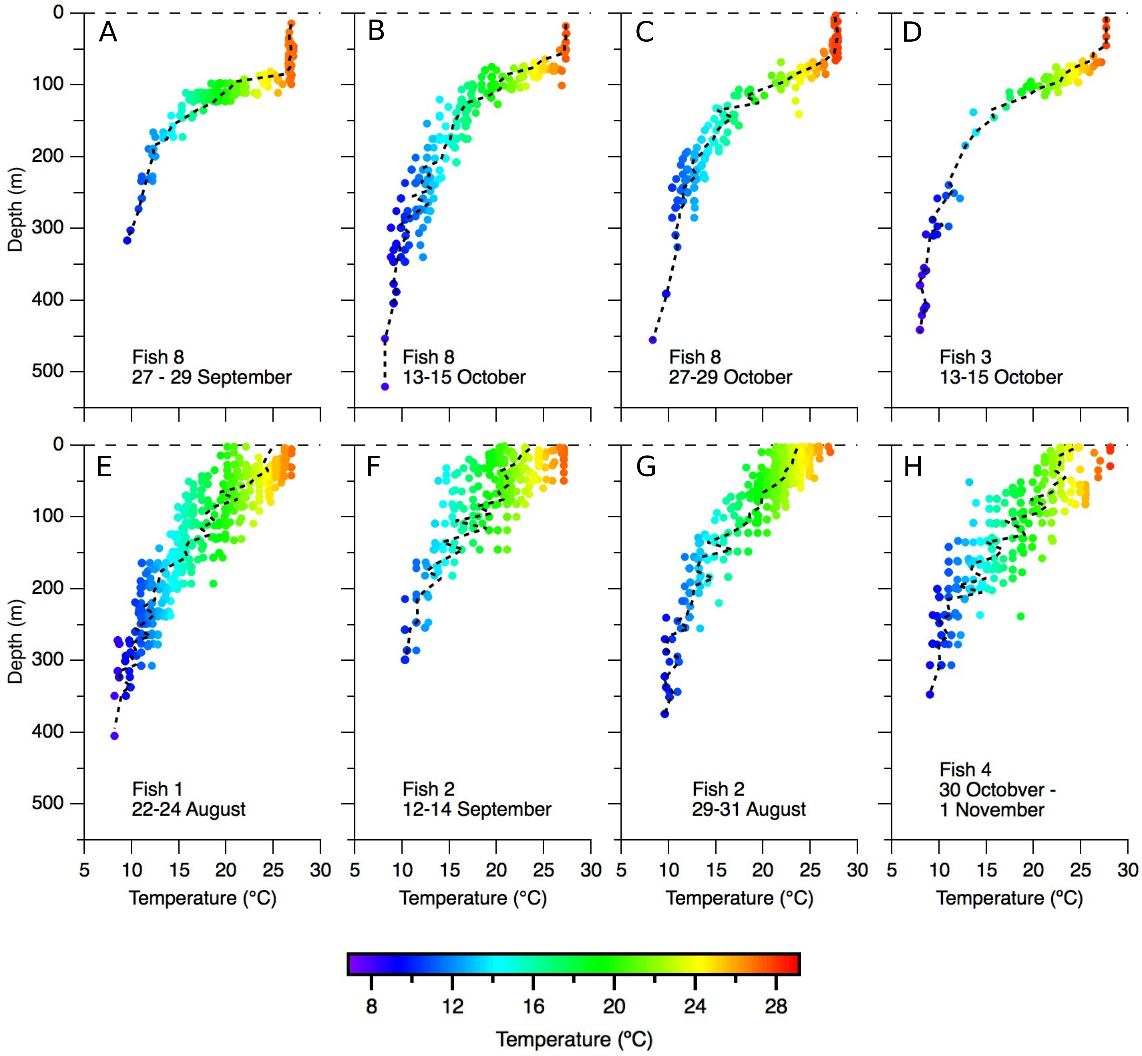


Figure 4.11 48-h depth – temperature profiles recorded north of the Lombok Strait (upper panel), and inside the Lombok Strait (lower panel). Data was recorded in 5 min (Fish 1) or 7.5 min intervals (Fish 2, 4 and 8). Broken black lines are mean depth in 10 m depth intervals.

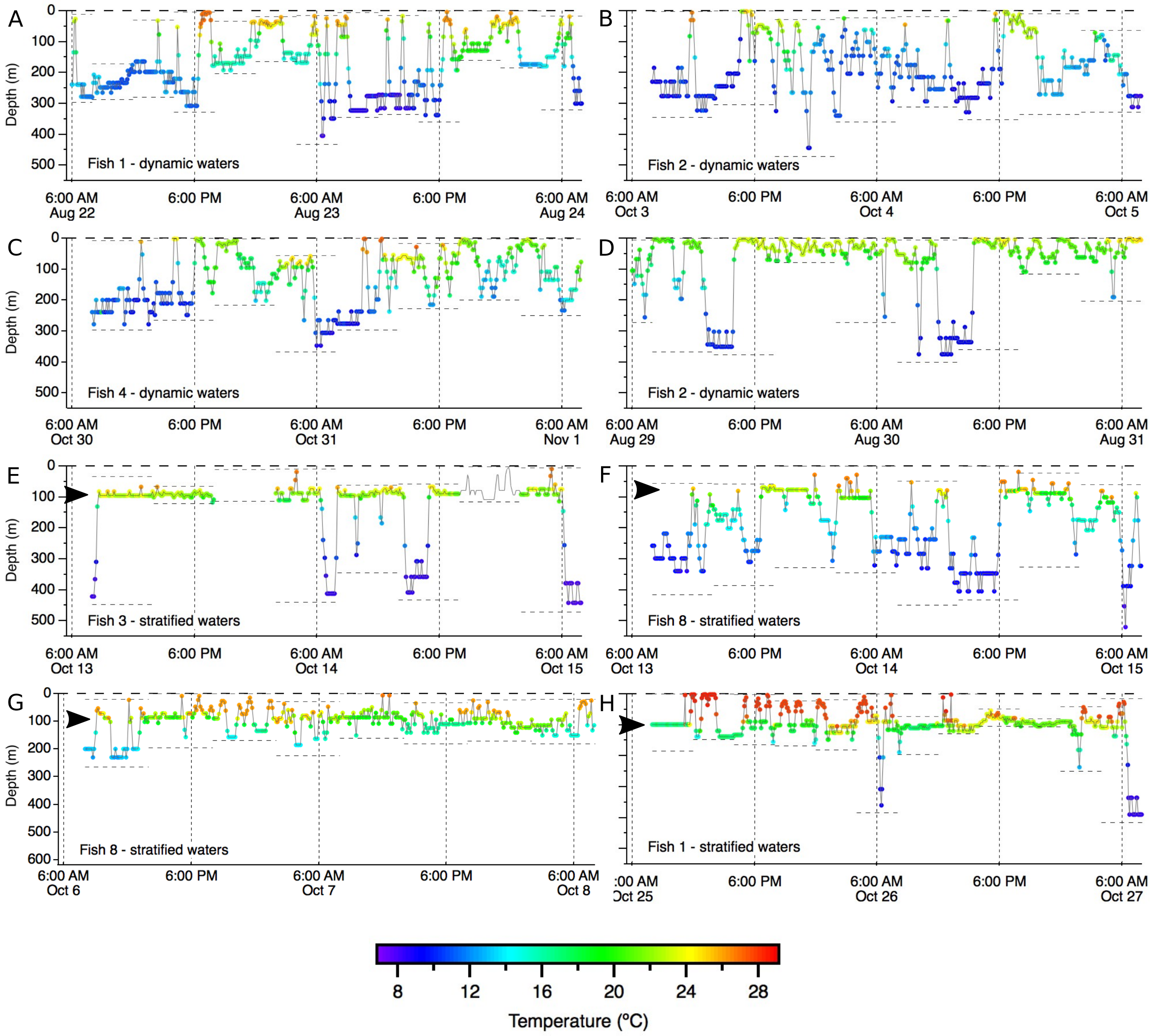


Figure 4.12 48-h depth and temperature (colour-coded) time series for fish in **A - D)** in dynamic waters of the Lombok Strait; and **E - H)** in stratified waters north of the Lombok Strait. Recording interval was 5 min (Fish 1) or 7.5 min (Fish 2, 3 and 8). Horizontal dashed lines are 6-h minimum and maximum depth values. Black triangles point to the ~100 m depth sunfish in stratified waters appeared to return to in between deep dives and surface visits.

4.5 Discussion

The overall aim of this study was to determine if temperature is likely the main driver of the sunfish seasonality on the Bali and Nusa Penida reefs. We approached this question by examining the timing of the 2015 sunfish season in relation to 1) the seasonal, large-scale changes in sea surface temperature in the Lombok Strait, 2) the timing of the seasonal cold-water upwelling, and 3) small-scale *in situ* temperature on the Nusa Penida reefs. Furthermore, we used Wildlife Computer SPLASH tags with Fastloc capabilities to examine the dive behaviour and depth occupancy of sunfish inside and outside the Lombok Strait, to determine if the Bali sunfish are likely to change dive behaviour when occupying warm, stratified waters compared with cooler, waters with volatile thermal structures. As our study provides the first detailed data on dive behaviour of *M. alexandrini*, we briefly discuss its diving and depth occupancy compared to other sunfish species.

4.5.1 The Bali sunfish season

The sunfish frequenting the Bali and Nusa Penida reefs appear to consist almost exclusively of *M. alexandrini*, which suggests that the seasonality of diver encounters with sunfish may be examined as a single species phenomenon. Analysis of dive tourism logs indicated that the 2015 sunfish season occurred during August – October/November, lagging the anecdotal sunfish season by ~1 month. Two oceanographic anomalies occurred in 2015, where an El Niño event was concurrent with a positive Indian Ocean Dipole (BOM 2017), however, these conditions would presumably have caused an early start to the sunfish season, rather than a delay (see further discussion in Section 4.5.4). Instead, the anecdotal sunfish season (July – September/October) coincided precisely with the diver high season found in this study (July – September/October 2015), indicating that the anecdotal sunfish season may have developed on the basis of tourism dive intensity (i.e. observational effort) rather than an increase in sunfish abundance on the reefs.

4.5.2 Spatial movements

During the 2015 sunfish season, four of the eight tagged *M. alexandrini*, with periods at liberty between 40 – 76 days, exhibited a high affinity to the oceanographically dynamic Lombok Strait, a small area of ~ 40 x 70 km. The high accuracy GPS Fastloc locations (10s to 100s of meters) revealed that the fish exhibited a particularly high affinity to the sill areas around Nusa Penida, and to the Bali coastal areas in the Western Lombok Strait. Seasonal residency in such a small coastal area concurrently by several tagged fish has not, to the best of our knowledge, been reported for sunfish elsewhere. Most sunfish telemetry research to date has targeted *M. mola* and has typically reported horizontal movements over much larger areas (100s – low 1000s km), including seasonal, latitudinal migrations, and has typically relied on light-based geolocations (Hays et al. 2009; Dewar et al. 2010; Potter et al. 2011; Thys et al. 2015). Interestingly, a study relying on Argos and GPS Fastloc locations in the northeast Atlantic showed that, in addition to north-south seasonal movements, sunfish spent extended periods in three ‘focal areas’, which included the Alboran Gyre and the Gulf of Cadiz, areas which are dominated by intense frontal activity associated with upwelling and relaxation events (Sousa et al. 2016b,c). Our findings were supported by a recent acoustic telemetry study of *M. alexandrini* off the Galapagos islands, where three of five tagged fish exhibited a high degree of site fidelity to a receiver station at Punta Vicente Roca, where they were each detected between 799 – 1,361 times during periods of 202 – 703 days, nearly year-round (Thys et al. 2017). At the time, these fish were identified as *M. ramsayi*, and later confirmed as *M. alexandrini* by (Sawai et al. 2018)

Not all tagged sunfish in the current study remained in the Lombok Strait. Two fish travelled north into the Bali sea immediately after tagging, where one tag detached prematurely after 17 days (yielding little data), while the other continued > 400 km north, where it exhibited a high affinity to the eastern edge of a sharp seabed gradient west of Pulau Kulakalukuang (100 – 500 m depth), south of the South Makassar Basin. Interestingly, this seabed gradient is associated with one of two main exit paths of the Indonesian Through Flow from the South Makassar Basin (Mayer and Damm 2012), and neighbours the superficially similar Dewakan sill; an area where extensive tidally-driven vertical mixing and internal waves create conditions akin to the Lombok Strait (Hatayama 2004). The remaining two tags yielded little data.

Existing information on the spatial movements of Bali sunfish is limited, consisting of four individuals tagged off Nusa Penida, which yielded tag pop-up locations but no track data (Thys et al. 2016). These fish were identified at the time as *M. ramsayi*, and later confirmation as *M. alexandrini* by (Sawai et al. 2018). Two of these tags were deployed in September 2004 and detached from the fish during October 2004, i.e. inside the sunfish season. Both tags popped up ~150 km south and south-southwest of Bali, respectively, in > 2,000 m of water. This is inconsistent with the high affinity of the tagged sunfish in the current study to the relatively shallow sill and coastal areas in the Lombok Strait, and the sharp (but relatively shallow) bathymetric gradient west of Pulau Kulakalukuang. We note, however, that the 12-h water column thermal structures for Fish A on fig. 6 in Thys et al. (2016) are consistent with the dynamic conditions typical of the Lombok Strait during the southeast monsoon (SST < ~25°C, shallow, volatile thermoclines; (Mitnik et al. 2000; this study), and with brief periods spent in conditions typical of the areas immediately north (and probably also to the south), of the Lombok Strait (SST > ~25°C, stratified waters with ~100 m thermoclines (Susanto et al. 2007; this study).

4.5.3 Vertical movements

In this study, two tags with 12-h time-at-depth histogram settings revealed a predominantly normal diel vertical migration (nDVM) pattern (i.e. deeper during the day than night), as also reported for sunfish elsewhere (e.g. Cartamil and Lowe 2004; Dewar et al. 2010; Nakamura et al. 2015; Thys et al. 2015). Sunfish nDVM has previously been linked to nearly continuous feeding on diel vertical migrating prey (Hays et al. 2009; Sims et al. 2009b), however a recent study using accelerometers established that tagged *M. mola* off Japan were inactive at night (Nakamura et al. 2015), similar to the findings of relatively limited night-time dive activity of tagged *M. mola* off eastern USA (Dewar et al. 2010). In the current study, night-time dive patterns generally revealed less dive activity than during daytime, but were occasionally reminiscent of daytime dive behaviour with repeated and/or sustained (h) dives below the thermocline. While night-time dive activities could potentially reflect sunfish predator avoidance behaviour, as suggested by Cartamil and Lowe (2004), it seems likely that in this case some degree of night-time feeding occurred.

The daytime dive profiles of our tagged *M. alexandrini* differed from the dive patterns reported for *M. mola* (Cartamil and Lowe 2004; Dewar et al. 2010; Nakamura et al. 2015), with regular yo-yo dives interspersed with frequent surface visits. Instead, our tagged fish spent extended periods at depth, visiting near-surface waters irregularly and oftentimes only briefly. Contrary to *M. mola*, which is infamous for its surface basking behaviour (e.g. Abe and Sekiguchi 2012), the low surface occupancy of tagged *M. alexandrini* in this study were more akin to that found for a tagged *Ma. lanceolatus* in the Gulf of Mexico, and much less than what is typically reported for *M. mola*, in the Atlantic (Sims et al. 2009b; Potter and Howell 2010; Sousa et al. 2016b), the Pacific (Cartamil and Lowe 2004; Dewar et al. 2010; Nakamura et al. 2015; Thys et al. 2015) and off South Africa (Hays et al. 2009). This may reflect species differences, alternatively, sea surface basking could potentially be a strategy in some or all the large Molidae species to achieve thermoregulation in the colder regions of their distribution ranges, as suggested for Atlantic *M. mola* by (Frafjord et al. 2017).

4.5.4 Shift in depth occupancy

Individual sunfish dive behaviour and depth occupancy is likely influenced by a combination of several factors at any given time, including food availability (e.g. sunfish prey densities, and search patterns related to prey patchiness (Sims et al. 2009b; Sousa et al. 2016b,c), thermoregulation (e.g. thermal structure of the water column, and temperature of the mixed layer in relation to sunfish body mass (Potter and Howell 2010; Nakamura et al. 2015), hypoxic conditions below the thermocline (Cartamil and Lowe 2004; Thys et al. 2015), and parasite removal by other animals such as cleaner fish (Konow et al. 2006; Thys et al. 2017) and seabirds (Abe and Sekiguchi 2012; Abe et al. 2012). However, in a recent study, (Sousa et al. 2016b) did not find significant relationships between *M. mola* depth occupancy and different types of water column stratification (stratified, mixed and frontal waters).

To examine the potential influence of water column stratification on the dive behaviour and depth occupancy of the Bali sunfish, data from fish occupying the dynamic waters of the Lombok Strait were compared with fish occupying stratified waters. Fish 8, which travelled > 400 km north and spent virtually the entire tagging period in stratified waters, spent significantly less time in the upper 50 m (daytime and night-time combined) compared with the fish which remained in the Lombok Strait (Fish 1 – 4) . This difference was also evident in the night-time (12-h) time-at-depth data between Fish 8 and Fish 1. These results indicate that Fish 8 responded to the water column stratification by limiting its exposure to the warm mixed layer. The daytime depth occupancy for Fish 8 was more complex, as a marked shift occurred on October 9, as the fish reached the steep seabed gradient west of Pulau Kulakalukuang. Prior to this date the fish appeared to be travelling, mainly in waters < 150 m. This was followed by a period of daytime occupancy mainly below 200 m and in temperatures of mainly 7 – 15°C, presumably reflecting foraging behaviour. As the water column stratification was similar during both periods, the shift was probably a behavioural response to favourable foraging conditions. A downwards shift in sunfish depth occupancy was also reported for *M. mola* travelling south along the east coast of the USA, into warmer, weakly stratified waters with a deep mixed layer (Potter and Howell 2010). The authors similarly speculated that the shift was linked to sunfish thermal preference or sunfish prey density.

The recurrent 48-h dive profiles revealed differing dive behaviour between fish in stratified versus dynamic waters. Specifically, Fish 8, which occupied stratified waters for nearly all of the tagging duration, tended to spend time near the thermocline (~100 m), a behaviour not evident in fish occupying the dynamic waters in the Lombok Strait. This behaviour was replicated in other fish during brief periods in stratified waters (i.e. black arrows on Figure 4.12E – H). It is not possible to say if this behaviour reflected the fish limiting their exposure to the warmer temperatures in the mixed layer above the deep thermocline, or if the deep thermocline conversely allowed the fish to thermoregulate in the lower part of the mixed layer, rather than closer to the surface, thereby saving vertical travel time in between deep foraging dives below the thermocline (e.g. Nakamura et al. 2015). At night, a deep thermocline may have allowed the fish to remain deeper, possibly reducing the risk of predator encounters (e.g. Cartamil and Lowe 2004). Regardless, this flexibility in dive behaviour may allow *M. alexandrini* (and other sunfish species) to extend their geographical distribution range into areas, which would otherwise have been beyond the upper thermal thresholds.

4.5.5 Upwelling chasers or thermal regulators?

The marked seasonality in sunfish abundance on the Bali and Nusa Penida reefs, as observed by tourist divers, is intriguing. Does it reflect a seasonal change in sunfish abundance in the Lombok Strait, or a change in sunfish visibility to tourist divers through a shift in sunfish depth occupancy between seasons, whereby the sunfish remain in deeper waters in the Lombok Strait during warm surface conditions, as suggested by Thys et al. (2016)? Existing tagging data outside the sunfish season is scarce, consisting of pop-up locations for two *M. alexandrini* tagged off Nusa Penida in October 2008 (Thys et al. 2016). One tag surfaced after six months (April 2009) < 10 km from the tagging site, while the other surfaced ~750 km east-southeast of Bali (January 2009) (*op. cit.*), supporting both scenarios.

Several studies have linked seasonal sunfish movements to changes in sea surface temperature (Potter et al. 2011; Thys et al. 2015; Sousa et al. 2016b). Specifically, Sousa et al. (2016b) and Thys et al. (2015) reported that tagged *M. mola* in the east Atlantic and off California, respectively, appeared to avoid SST ≥ 25°C through seasonal latitudinal movements. Similarly, global distribution modelling recently found an upper thermal threshold for *Mola* spp. of ~23°C (Phillips et al. 2017), while Nakamura and Sato (2014) found that the catches of *M. mola* in set nets off Japan declined sharply when temperatures exceeded 20°C. In line with these upper thermal thresholds, Thys (2016) suggested that the Bali sunfish may prefer deeper, cooler waters of 20 – 24°C during the northwest monsoon. However, thermal tolerances appear to differ between *Mola* species, and perhaps even between genetic clades within species (Yoshita et al. 2009; Nyegaard et al. 2018a) [Chapter 3]. The findings in this study indicate that the thermal tolerance for *M. alexandrini* is higher than for *M. mola*, in that it includes exposure to 27 – 29°C waters, similar to the findings by Thys et al. (2016) for one of the *M. alexandrini* previously tagged off Nusa Penida. Other studies have found *M. alexandrini* to be associated with higher SSTs than both Pacific *M. mola* and *M. tecta* (Sawai et al. 2011; Nyegaard et al. 2018a) [Chapter 3], and *M. alexandrini* appears to have a lower latitude distribution worldwide than *M. mola* (Sawai et al. 2018). Nevertheless, it is possible that the warm conditions in the Lombok Strait during the northwest monsoon and transitional months, where the sea surface can surpass 29°C as the Indonesian Through Flow weakens (Figure 4.3, Figure 4.4A,B), exceeds the upper thermal threshold of *M. alexandrini*.

This may in turn cause a downwards shift in depth occupancy akin to that exhibited by the tagged fish in this study when they occupied stratified waters.

Our findings, however, suggest that such a temperature driven shift in vertical distribution between seasons is an unlikely driver of sunfish abundance on the Bali and Nusa Penida reefs. Specifically, we found a ~1 month lag between the drop in sea surface temperature (July 2015) associated with the southeast monsoon, and the onset of the sunfish season (August 2015). This is inconsistent with a resident sunfish population avoiding warm surface waters. Furthermore, the *in situ* temperature loggers at 30 m on several Nusa Penida reef sites revealed that even when the SST was high, small-scale vertical mixing produced sub-surface temperature regimes well within the *M. alexandrini* thermal tolerance, during months where sunfish encounter rates were low. This was particularly the case at the popular sunfish site Crystal Bay during April – July.

The anecdotal link between sunfish sightings and cold water off Nusa Penida may instead have developed due to an indirect relationship between sunfish abundance and low temperatures. The seasonal drop in SST coincides with the cold-water upwelling along the south coast of Bali and Nusa Penida. This seasonal upwelling is known to trigger an increase in primary production (Hendiarti et al. 2004; Gordon 2005; Susanto et al. 2006), a well-known phenomenon in upwelling systems across the world (Kämpf and Chapman 2016). Possibly, this increase in primary productivity causes an increased availability of prey for *M. alexandrini*, attracting sunfish to the area.

The prey of *M. alexandrini* is not known but probably includes gelatinous zooplankton, as has been reported for 'larger' *M. mola* (> ~80 – 100 cm TL: siphonophores, scyphozoans and ctenophores [Nakamura and Sato 2014; Nakamura et al. 2015] and hydrozoans [Sousa et al. 2016a], and for *M. tecta* (salps and siphonophores; Nyegaard et al. 2018b) [Chapter 2]. Little is known of the gelatinous zooplankton diversity, distribution and abundance in the Lombok Strait and in the Java upwelling system. However, gelatinous zooplankton are generally capable of rapid reactions to favourable environmental conditions through various life cycle and reproductive strategies, creating blooms through rapid population growth (Boreo et al. 2008; Henschke et al. 2016). Gelatinous zooplankton abundance may also increase in upwelling areas from aggregation due to physical processes (e.g. Graham et al. 2001). A link between an increase in gelatinous zooplankton abundance and upwelling has been found in other parts of the world including the Bay of Panama and Monetary Bay (Robison et al. 1998; Miglietta et al. 2008). Specifically, Robison (1998) found that the small siphonophore *Nanomia bijuga* was predominantly concentrated between 200 and 400 m depth, and that their peak abundance occurred approximately three months after the onset of the upwelling. Similarly in the Bali Strait, between Java and Bali, Sartimbul et al. (2010) examined catch per unit effort of the small teleost 'Bali sardinella' (*Sardinella lemuru*) and found that peak catch rates were found three months after the seasonal increase in Chl-a associated with the seasonal upwelling. They postulated this represented the lag time for trophic energy transfer from phytoplankton to *S. lemuru*, presumably through the crustacean zooplankton pathway (*op. cit.*). Similarly, the start of the 2015 sunfish season (August) found in the current study lagged the start of the upwelling season (July) by ~1 month, with sunfish sightings rates peaking in September, possibly reflecting a lag in trophic level energy transfer between primary producers and sunfish prey (presumably gelatinous zooplankton). This may also explain the tail-end of the sunfish season lasting into November, despite the decreasing upwelling conditions.

Overall, our findings indicate that the Lombok Strait may be a seasonal foraging area for the Bali sunfish, with at least four of our tagged fish exhibiting a high affinity to this small area during their time at liberty. Other telemetry and observational studies of *M. mola* and *M. alexandrini* have likewise shown that sunfish seek out fronts and upwelling systems where productivity is high (e.g. Sims and Southall 2002; Thys et al. 2015; Sousa et al. 2016b; Halhlbeck et al. 2017). Interestingly, the seasonal cold-water upwelling off Bali occurs over a large area, along the southern coastlines of Java and other neighbouring islands (Gordon 2005; Susanto and Marra 2005; Ningsih et al. 2013). Nevertheless, the tagged sunfish in this study did not appear to frequent these areas. It is possible that the specific conditions in the Lombok Strait, with strong tidal currents over complex topography, extensive tidally driven vertical mixing and propagation of internal waves, generate an advantageous feeding location for sunfish, perhaps akin to 'trophic focusing' (Genin 2004). In this context, the affinity of Fish 8 to the potentially similar area south of the South Makassar Strait is interesting, as it indicates that similar small-scale 'hot-spots' of favourable foraging areas for sunfish could exist elsewhere in the oceanographically diverse and dynamic Indonesian seas (Gordon 2005; Gordon et al. 2010b; Nagai and Hibiya 2015).

In 2015, there was a general perception among Nusa Lembongan dive operators that above average numbers of sunfish were present on the Nusa Penida reefs (S Faust, All4Diving; A Taylor, Blue Corner Dive; S Bebe, World Diving, pers comm 2018). The 2015 anomaly conditions of a concurrent El Niño and a positive Indian Ocean Dipole are associated with particularly strong upwelling and increased primary production along the southern coast of Bali and neighbouring islands (Hendiarti et al. 2004; Susanto and Marra 2005; Ningsih et al.

2013). This seemingly supports the notion that the Bali sunfish seasonality is related to an increase in food availability associated with the seasonal cold-water upwelling. However, further research is needed to investigate inter-annual variation in the occurrence of the sunfish season, and potential variation in abundance between years.

4.5.6 Early tag dislodgement

All our tags detached prematurely due to dart dislodgement, probably associated with tether fouling with the dorsal fins during cleaner-fish interactions, where sunfish angle their head upwards (Figure A4.1.5 in Appendix A4). The shorter tether (50 cm) on Fish 1 yielded the longest deployment period (76 days) compared with the longer tethers (100 cm) on Fish 2 – 4 and Fish 8 (17 – 62 days; mean ± SE = 47.5 ± 8.3), however it also had the lowest GPS acquisition rate (Section 4.4.4; Table 4.1).

Overall, GPS Fastloc is a powerful tool for sunfish movement ecology (e.g. Sims et al. 2009a; Sousa et al. 2016b; Thomson et al. 2017), and proved critical in this study to reveal high sunfish occupancy inside the Lombok Strait during the sunfish season, as well as the high affinity of one fish to the steep seabed gradient south of the South Makassar Basin. However, longer term deployments of towed SPLASH tags will require a more robust attachment method than used in this study, which would be achievable during brief capture and confinement of sunfish (e.g. Houghton et al. 2009; Nakamura et al. 2015; Sousa et al. 2016b). Unfortunately, sunfish capture and confinement is not straight forward in the Nusa Penida MPA due to the low surface occupancy of the Bali sunfish, and improvements to tag attachment should ideally be achievable during brief sunfish encounters by SCUBA divers under challenging dive conditions.

4.5.7 Conclusions

The rapidly increasing SCUBA tourism within the Nusa Penida MPA is currently causing intense diver pressure on several Nusa Penida MPA reefs, including the popular sunfish dive site Crystal Bay in the South Channel. The wide use of the Lombok Strait by four tagged sunfish, suggest extensive opportunities exists here for cleaner-fish interactions on little dived reefs, as well as at depths below recreational dive limits. Our study showed, however, that not all reefs are equally attractive to sunfish. The effort corrected encounter rates corroborated anecdotal knowledge that the chance to see sunfish are far better at some dive sites than others. This makes the known ‘sunfish sites’ important to protect and manage.

The sub-lethal impacts on individual sunfish and cleaner-fish populations from diver crowding are unknown, but the dive pressure on the Nusa Penida and Bali reef systems is palpable. It is possible that the biggest threat to the Bali sunfish phenomenon lies in degradation of the coral reefs, and associated impacts to relevant cleaner fish assemblages. This includes mechanical damage from high dive pressure, as well as the tendency of some operators to take divers with poor buoyancy skills to the challenging Nusa Penida dive sites to see sunfish. The reefs along the Nusa Penida islands are relatively limited, with a coastline of < 100 km. While these reefs are to some extent protected from mass coral reef bleaching events by the seasonal cold-water upwelling and small-scale cold-water events from vertical tidal mixing, effective management is needed of the diver tourism, as well as the tourism in general, to ensure that the reefs remain attractive to sunfish and tourists alike in the future.

Table 4.1 Summary of satellite tagging of eight *Mola alexandrini* in the Lombok Strait, Indonesia, 2015, with SPLASH tags from Wildlife Computers). Italics denote tags which yielded very limited data due to early release and/or transmission failure.

Fish	**Fish 1**	**Fish 2**[a]	**Fish 3**	**Fish 4**[a]	**Fish 5**	**Fish 6**	**Fish 7**[a]	**Fish 8**
Estimated Total Length (m)	1.4	1.6	1.5	1.5	*1.9*	*1.5*	*1.6*	1.6
Histogram setting (h)	12	24	24	24	*24*	*12*	*12*	12
Tag date (2015)	21 Aug	28 Aug	7 Sep	10 Sep	*13 Sep*	*19 Sep*	*20 Sep*	21 Sep
Tag detachment date (2015)	05 Nov	15 Oct	17 Oct	11 Nov	*13 Sep*	*6 Oct*	*21 Sep*	31 Oct
Days at liberty (days)	76.5	48.4	40.3	62.0	*0.6*	*17.2*	*1.6*	40.5
Distance between tagging and tag pop-off locations (km)	29.6	24.2	63.0	21.4	*18.7*	*119.9*	*20.5*	327.4
Max recorded depth (m) ± sensor accuracy	464 ± 16	472 ± 4	552 ± 8	464 ± 16	*269.5 ± 23*	*375 ± 25*	*375 ± 25*	552 ± 8
Total no. GPS Fastloc[c] (mean GPS.day^{-1})	8 (0.1)	40 (0.8)	19 (0.5)	16 (0.3)	*1 (n/a)*	*1 (0.1)*	*0 (n/a)*	12 (0.3)
Total no. of Argos positions	0	6	3	3	*0*	*1*	*0*	5
Highest minimum horizontal speed between Fastloc positions (m/s)	0.3	1.3	0.5	0.6	*1.2*	*0.2*	*0.1*	0.5
Days of tag transmission post-detachment	49.4	15.9	35.5	6.6	*1.3*	*0.3*	*16.8*	28.1
Complete 24-hr TAD sets recovered	73 (97%)	42 (89%)	39 (99%)	22 (36%)	*n/a*	*2 (12%)*	*n/a*	37 (94%)
Complete 24-h TAT sets recovered	72 (95%)	42 (89%)	39 (99%)	23 (38%)	*n/a*	*2 (12%)*	*n/a*	37 (94%)
Complete (partial) 48-h depth and temperature time series recovered	5 (0)	6 (1)	0 (2)	2 (0)	*0 (1)*	*0 (2)*	*0 (1)*	4 (2)

TAD: Time at Depth; TAT: Time and Temperature; n/a: not applicable; m/s: meters per second

[a] Tissue sampled

[b] From sea surface temperature or time series data.

[c] Excluding the first GPS position after tag detachment (i.e. tag 'pop-up' location)

[d] Discounting first and last (incomplete) days at liberty

Table 4.2 Results of statistical analyses.

Comparison of:	Histogram setting	Median 1	Median 2	Mann-Whitney 2-tailed test
Median TAD (0 – 50 m)	24 h	19.8% (Fish 1 – 4 combined)	5.75% (Fish 8)	$U_{176,37} = 5{,}401$, $p < 0.01$
Median TAT (27 – 29°C)	24 h	0.6% (Fish 1 – 4 combined)	4.8% (Fish 8)	$U_{176,37} = 1{,}424.5$, $p < 0.01$
Daytime TAD (> 200 m)	12 h	16.3% (17.0%[a]) (Fish 8 before 9 Oct)	74.1% (Fish 8 after 9 Oct)	$U_{16,21} = 7$, $p < 0.01$ ($U_{13,21} = 3$, $p < 0.01$)*
Daytime TAT (< 15°C)	12 h	20.8% (Fish 8 before 9 Oct)	80.6% (Fish 8 after 9 Oct)	$U_{16,21} = 4$, $p < 0.01$
Night-time TAD (0 – 50 m)	12 h	11.7% / 6.3% (Fish 8 before/after 9 Oct)	27.2% (Fish 1)	$U_{16,73} = 957.5$, $p < 0.01$ / $U_{21,73} = 1{,}376$, $p < 0.01$
Night-time TAT (27 – 29°C)	12 h	4% / 7% (Fish 8 before/after 9 Oct)	0.5% (Fish 1)	$U_{16,73} = 346$, $p < 0.05$ / $U_{21,73} = 211$, $p < 0.01$

TAD: Time at Depth; TAT: Time and Temperature

[a] Excluding the first 72 h while Fish 8 was still inside the Lombok Strait

Appendix A4.1 Light-based geolocations

Table A4.1.1 Comparison of model scores using the Wildlife Computers online Global Position Estimator (GPE3) for Fish 2, varying the fish speed (1- 3 m/s). The model was provided with either tagging and pop-up seed locations only ('wo GPS'), or with tagging, GPS Fastloc and Argos seed locations ('w GPS'). In addition, the model was run either with sea surface temperature tag data ('w SST'), or without ('wo SST'). Model results are shown in Figure A4.1.1.

	GPE3 model scores	
Animal speed (m/s)	***wo GPS, w SST***	***wo GPS, wo SST***
1	18.19	15.06
2	20.70	16.48
3	22.65	17.29
Animal speed (m/s)	***w GPS, w SST***	***w GPS, wo SST***
1	25.92	28.12
2	27.61	29.14
3	28.13	29.95

Table A4.1.2 Comparison of model scores using the Wildlife Computers online Global Position Estimator (GPE3) for fish with tag retention > 48 h (Fish 1, 3, 4, 6 and 8) varying the fish speed (1- 3 m/s). The model was provided with tagging, GPS Fastloc and Argos seed locations ('w GPS'), and was run either with sea surface temperature tag data ('w SST'), or without ('wo SST').

		GPE3 model scores		
	Animal speed (m/s)	***w GPS, w SST***	***w GPS, wo SST***	***Figure reference***
Fish 1	1	18.19	15.06	Figure A4.1.2
	2	20.70	16.48	
	3	22.65	17.29	
Fish 3	1	19.25	10.76	Figure A4.1.3
	2	19.65	11.12	
	3	19.83	11.52	
Fish 4	1	16.74	13.91	Figure A4.1.3
	2	18.83	14.76	
	3	19.45	14.85	
Fish 6	1	32.73	27.78	Figure A4.1.3
	2	34.28	28.16	
	3	35.30	27.97	
Fish 8	1	27.25	14.32	Figure A4.1.3
	2	27.81	15.57	
	3	28.01	15.82	

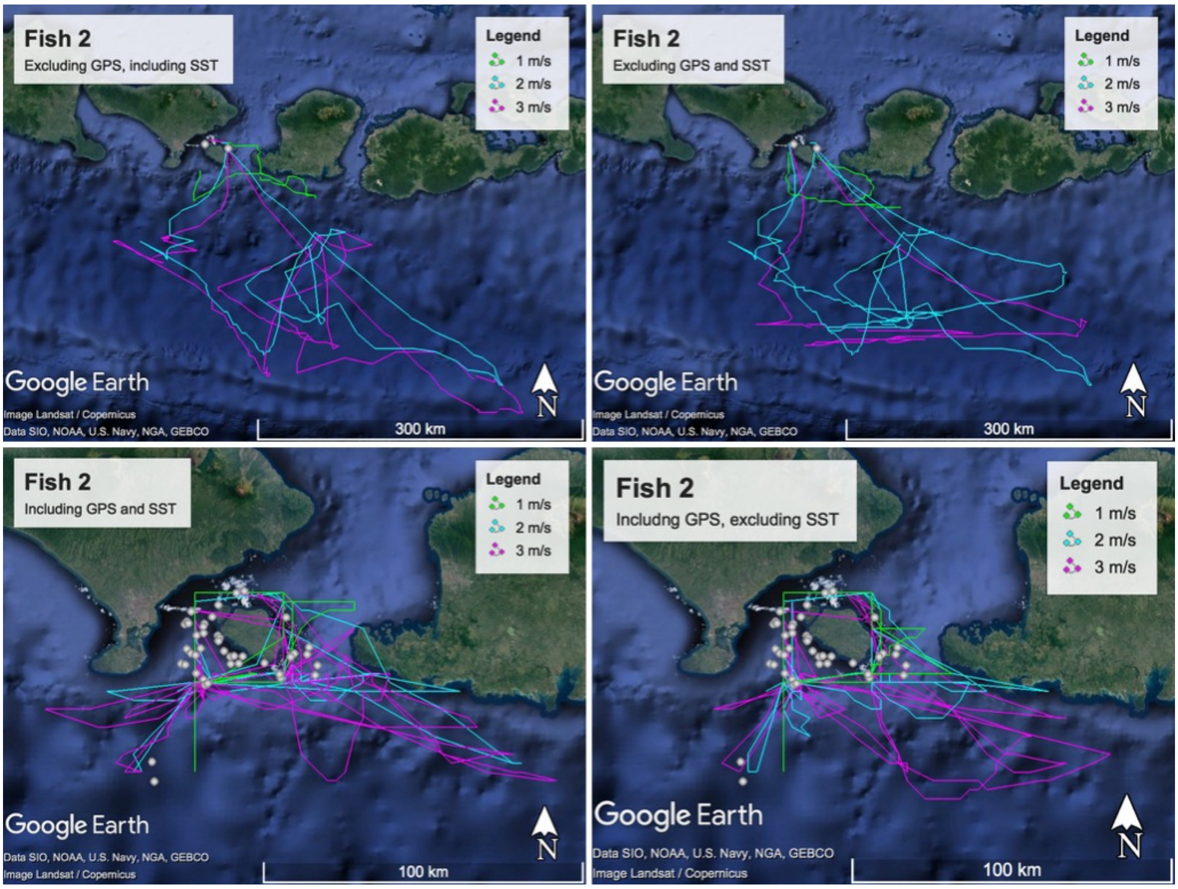


Figure A4.1.1 Light-based geolocation maximum GPE3 model output for Fish 2 (refer to Table A4.1.1 for details). White circles are the seed locations (tagging, GPS Fastloc and Argos locations) included in the model.

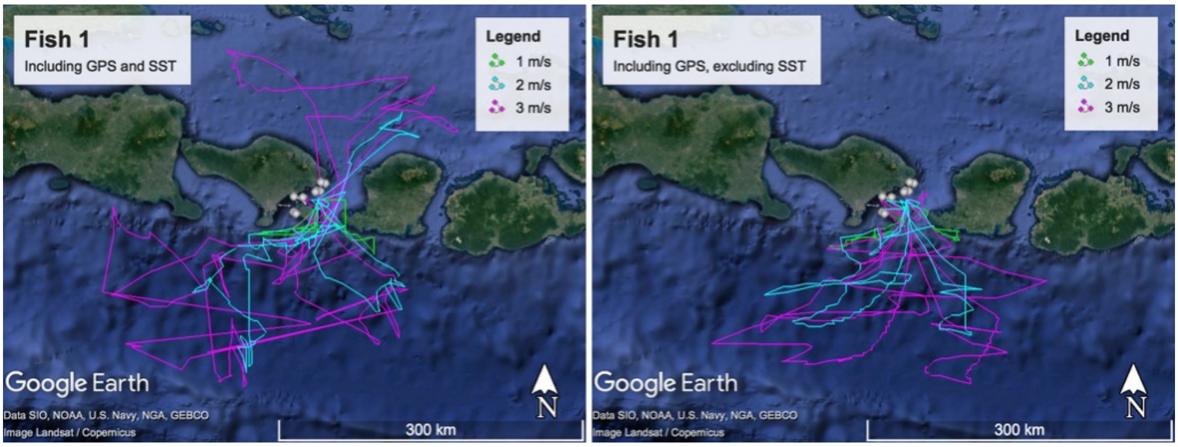


Figure A4.1.2 Light-based geolocation maximum likelihood GPE3 model output for Fish 1, 3 and 4– 3 (refer to A4.1.2 for details). White circles are seed locations (tagging, GPS Fastloc and Argos locations) included in the model.

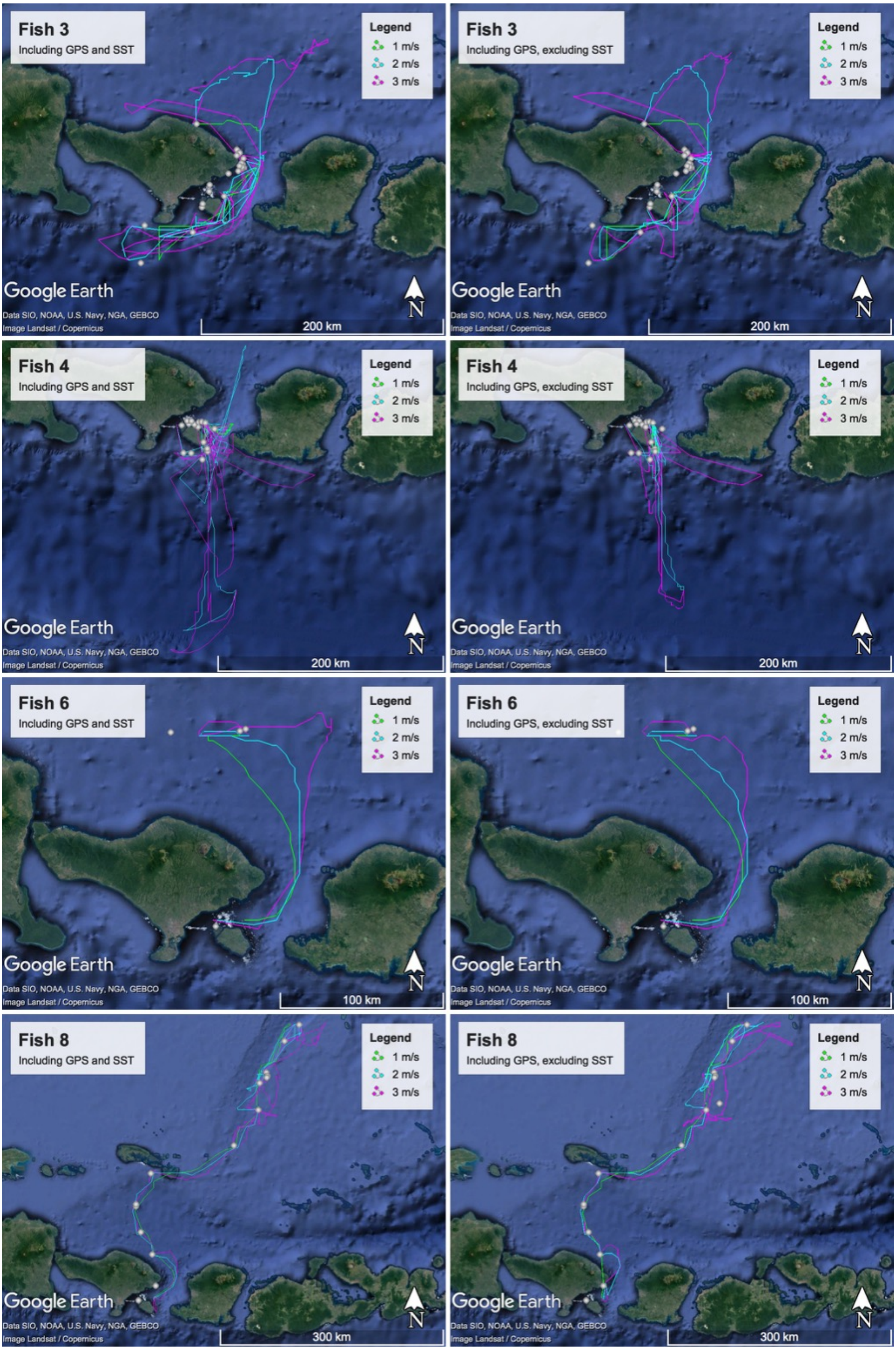


Figure A4.1.3 Light-based geolocation maximum GPE3 model output Fish 6 and 8 (refer to A4.1.2 for details). White circles are seed locations (tagging, GPS Fastloc and Argos locations) included in the model.

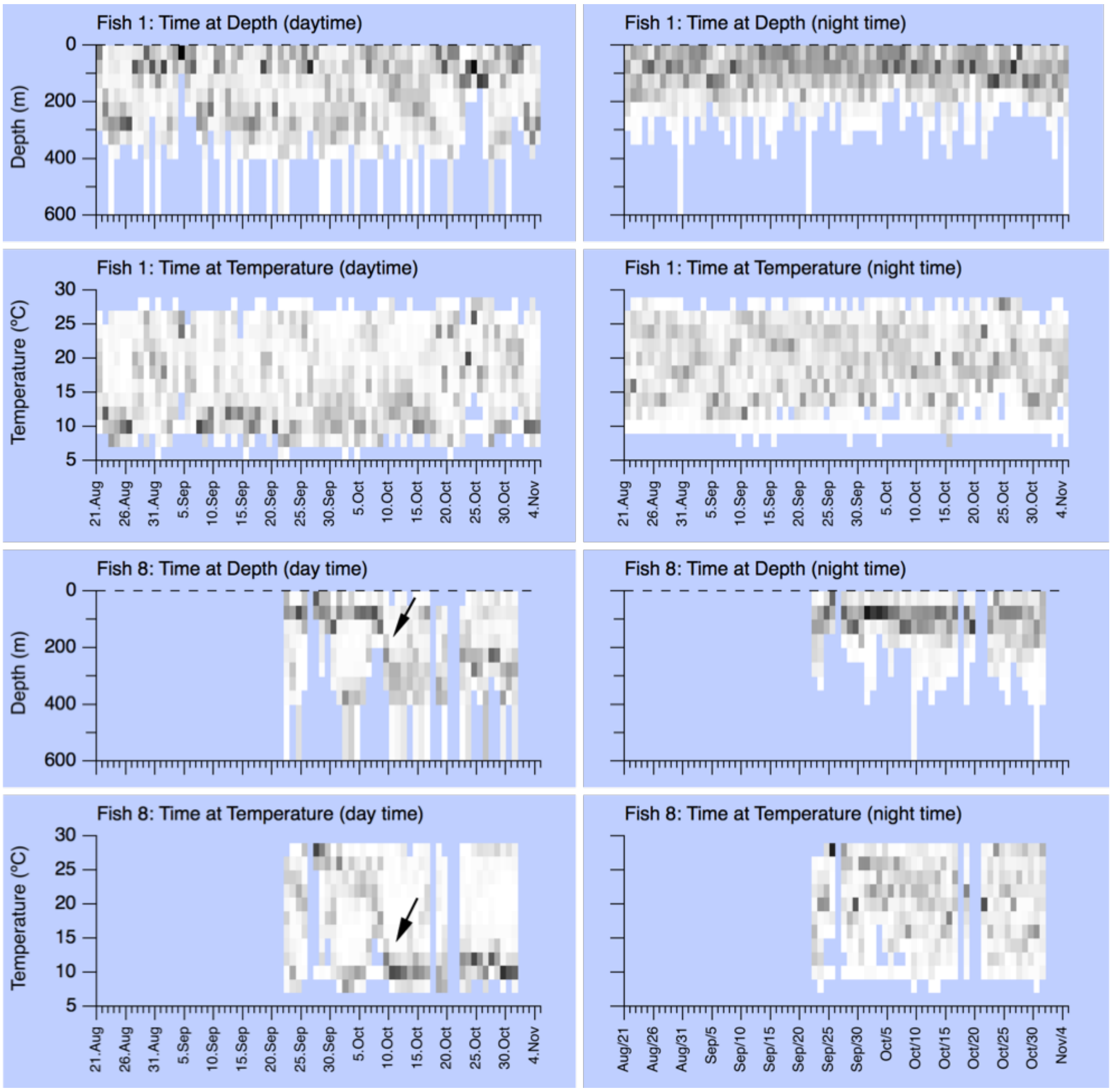


Figure A4.1.4 Daily (left panel) and nightly (right panel) percent time spent at depth and temperature in 12 h intervals for Fish 1 (upper four figures) and Fish 8 (lower four figures) during the 2015 tagging program. Arrows denote the shift in depth occupancy and temperature exposure for Fish 8 around October 9 2015.

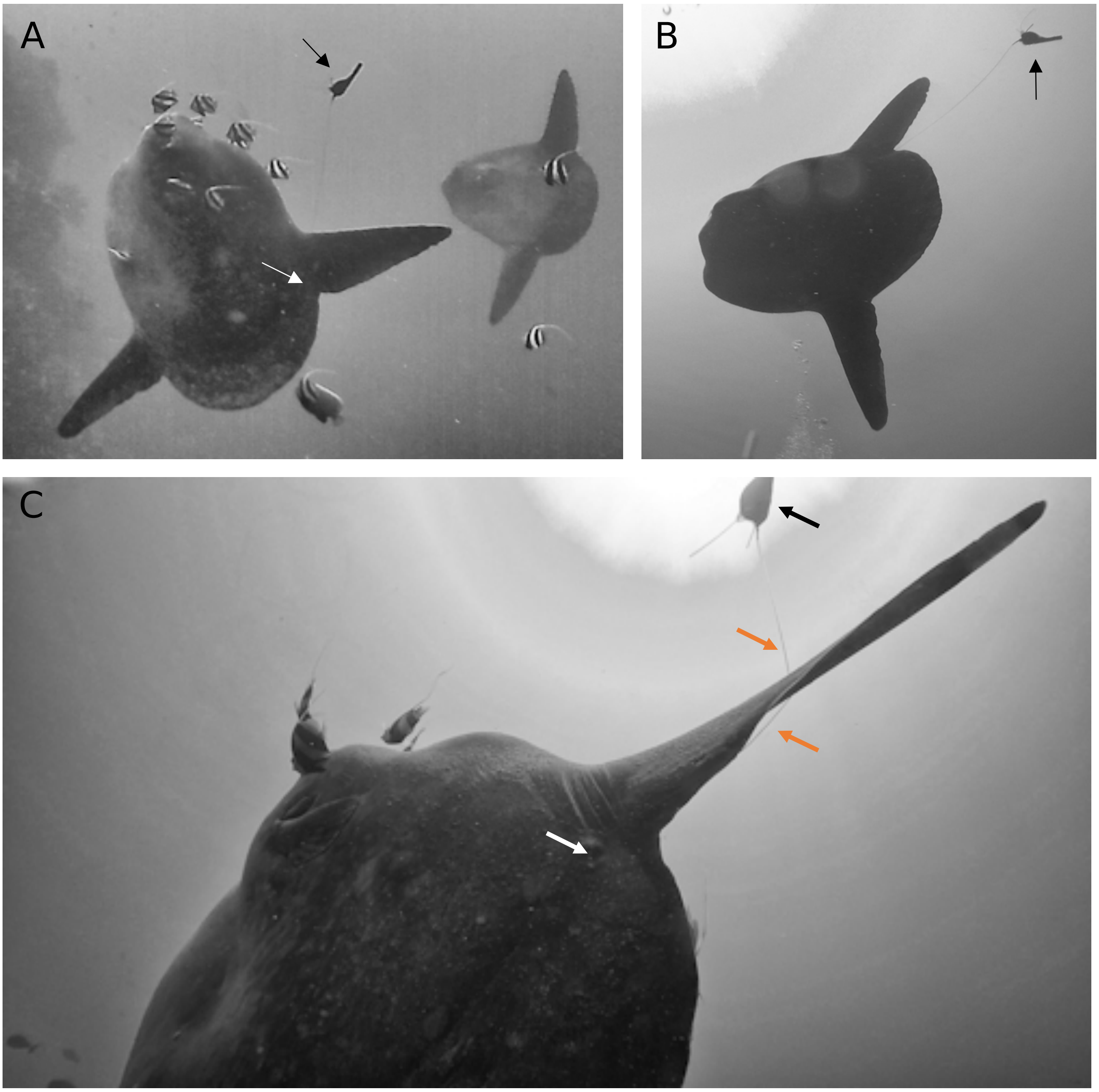


Figure A4.1.5 Tagging position (white arrows) just laterally of the dorsal fin, and behaviour of the tag (black arrows) during **A,C)** sunfish – cleaner-fish interactions and **B)** swimming. Orange arrows indicate fouling of the tag tether with the sunfish' dorsal fin.

Chapter 5 General conclusions

5.1 Summary of findings

At the inception my PhD candidature, genetic research indicated that *Mola* taxonomy was in need of a review, and that sunfish diversity and zoogeography had potentially been confounded by a long legacy of taxonomic confusion. In my research, I aimed to gain clarity on which species of large ocean sunfishes occur in the Oceania region, to establish how anthropogenic pressures may be assessed on a species level. The anthropogenic pressures I looked at were sunfish bycatch in the longline fisheries in Australia and New Zealand, and SCUBA diver crowding of sunfish in the tourism industry in the Bali area, Indonesia.

A number of important findings arose from my PhD research. These are summarised below, and discussed further in Sections 5.2.1 – 5.2.3.

- I found and described *Mola* sp. C and resolved the nomenclatorial status, resulting in the first addition of a valid species to the genus *Mola* in nearly 180 years, *M. tecta* (Chapter 2).
- Contrary to long-standing belief, I found that *M. mola* is not the dominant sunfish species in Australia and New Zealand, instead *Ma. lanceolatus*, *M. alexandrini* and *M. tecta* are found in the tropical, subtropical/warm-temperature and cold-temperate regions, respectively, of the southwest Pacific (Chapter 3).
- Taking this new information on sunfish zoogeography into consideration, I undertook the first large-scale review of sunfish longline bycatch in Australia and New Zealand and found that the annual interactions were overall relatively modest in scale, with no evidence of declining trends in bycatch rates over time (Chapter 3).
- Based on new taxonomic clarity, I verified the species identity of the Bali sunfish, *M. alexandrini* and confirmed the occurrence of sunfish on the Bali reefs can be considered as a single-species phenomenon (Chapter 4).
- I provided the first data-based estimate of the sunfish seasonality on the Bali reefs, and found that during the sunfish season some individuals were seasonal residents of the Lombok Strait, while others wandered more widely (Chapter 4).
- The seasonal presence of sunfish on the Bali reefs is likely a reflection of seasonal changes in sunfish abundance in the Lombok Strait, linked to seasonal cold-water upwelling and prey availability.

5.2 On the importance of taxonomic clarity for sunfish research

5.2.1 Resolving *Mola sp. C*

During my research, and through collaborative efforts with Japanese and New Zealand taxonomists, I found, described and named *Mola* sp. C as a new species, *Mola tecta*. Concurrently, the Japanese research group was working on resolving *Mola* sp. A, and eventually equated this species clade with *M. alexandrini*, a senior synonym of *M. ramsayi* (Sawai et al. 2018).

Our research revealed that *M. alexandrini* and *M. tecta* have very similar body morphologies at small sizes (e.g. ~ < 65 – 70 cm total length) (e.g. Figure 5.1) and that close examination or genetic analysis is necessary to distinguish the two species at these small sizes. The species characteristics develop as the sunfish increase in size. This includes differing scale morphology and clavus meristics as well as a marked difference in body shape (Sawai et al. 2018) (Figure 5.1). The latter provides the means to verify species through photographs and direct observations

Combined, our research provided the first update to the genus *Mola* since Fraser-Brunner (1951), which now consists of the ocean sunfish *M. mola* (Linnaeus, 1758), the bump-head sunfish *M. alexandrini* (Ranzani 1839), and the hoodwinker sunfish *M. tecta* Nyegaard et al. 2017 (Eschmeyer et al. 2018).

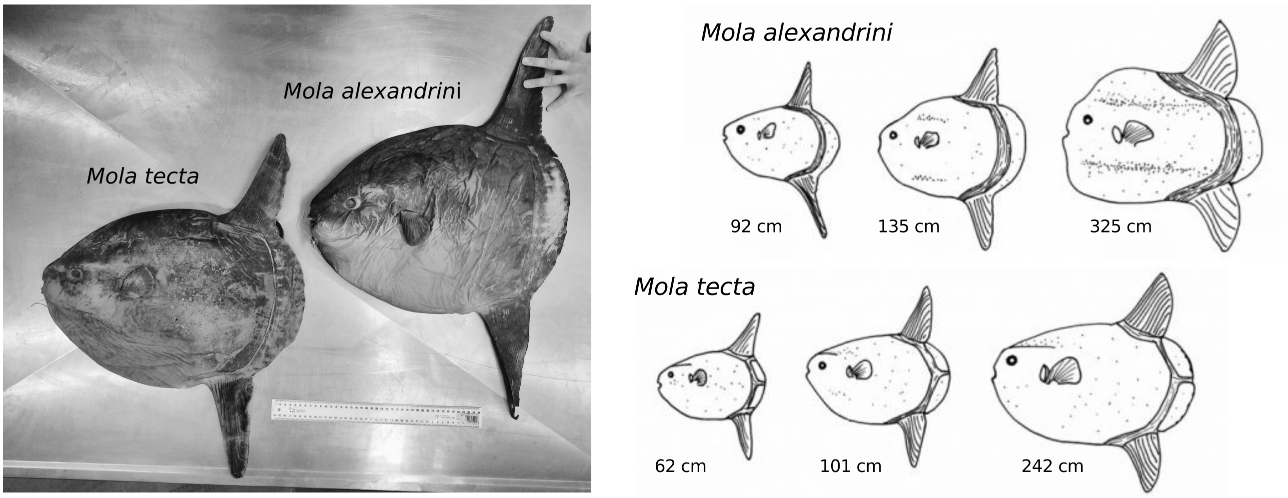


Figure 5.1 Left: *Mola tecta* (specimen NMNZ P.5890) and *M. alexandrini* (specimen NMNZ P.034449), both 51.5 cm total length. Right: change in body morphology of *M. tecta* and *M. alexandrini* with increasing size. Photo by E Sawai; illustration by E Burns, based on illustrations by M Freeborn (Nyegaard et al. 2018b [Chapter 2]) and E Sawai (Sawai et al 2017).

5.2.2 Sunfishes in the Australia and New Zealand longline fishery

Contrary to the belief at the inception of this research, my results strongly imply that *M. mola* is not the most common sunfish species in Australia and New Zealand. Instead *Ma. lanceolatus*, *M. alexandrini* and *M. tecta* each appear to be the dominant species of large ocean sunfish in the tropical, sub-tropical/warm-temperature and cold-temperate marine regions of Pacific Australia and New Zealand, respectively (Chapter 3).

With renewed clarity on the species-level distribution of sunfishes in Australia and New Zealand, including the extent of historic and current confusion between species, it became clear that assessing species-level bycatch trends of Australian and New Zealand sunfish was not straight forward. Bycatch rates were examined in sub-areas of the fishing grounds in an attempt to assess temporal trends in bycatch, each likely dominated by a single species. No discernible temporal trends in bycatch rates over time were found, which suggests that the longline fishery off east coast Australia and New Zealand probably does not, at the current levels of fishing effort, cause undue pressure on the sunfish populations.

On a broader scale, the magnitude of bycatch rates and total annual interactions in the longline fisheries off the east coast Australia and New Zealand were similar to those reported from the South African longline fishery (Peterson 2005). In Australia and New Zealand, total annual sunfish interactions were in the order of hundreds or low thousands. These results indicate that the risks to sunfish from the longline fishing industry in these areas are not as dramatic as suggested by the IUCN assessment of *M. mola* (Liu et al. 2015). Here, the IUCN cited an estimate of 340,000 annual interactions in the South African longline fishery as a warning sign the longline fishing method in general may be highly detrimental to sunfish populations worldwide (Liu et al. 2015). However, this estimate appears to have been based on an erroneous interpretation in Sims et al. (2009b) of the South African longline catch rates reported in Petersen (2005), where total annual interactions are in the hundreds (*op. cit.*).

Gauging the risk of the longline fishing method to sunfish populations worldwide is beyond the scope of this thesis. However, my results imply that all the large species of sunfish are probably caught in longline fisheries operating within their range, not just *M. mola*. Given the current uncertainties in the species-level zoogeography of the large sunfishes globally, it may be more practical to tentatively treat the global risk to sunfish populations from longline pressure as *Mola* spp. and *Ma. lanceolatus* combined. Given the extensive longline operations across the Worlds' oceans (Lewison et al. 2004; Kroodsma et al. 2018), it may be pertinent to investigate sunfish bycatch species compositions and post-release mortality (if released), to better understand the scale of the global longline bycatch pressure on each species.

5.2.3 Sunfish in the nature-based tourism industry in Bali, Indonesia

The increased taxonomic clarity in the genus *Mola* allowed me to confirm that the Bali sunfish appear to consist almost exclusively of *M. alexandrini* (Chapter 4). This was an important basis for using diver observational data and telemetry to examine the strong seasonality of the Bali sunfish as a single species.

The finding that the 2015 sunfish season in the Nusa Penida Marine Protected Area was offset from the anecdotal sunfish season by ~1 month was based on the first effort-corrected analysis of observational data for the area. Instead, the anecdotal sunfish season coincided with the 2015 dive tourism high season, indicating that the anecdotal season probably reflects dive tourism intensity (i.e. observational effort) rather than an increase in the underlying sunfish abundance. The implications for tourism risk management is that any future perception of 'failure' of the onset of the sunfish season, or a 'late beginning' does not necessarily reflect impacts associated with diver crowding.

The use of satellite tags with GPS Fastloc capabilities produced the first high-resolution movement data for the Bali sunfish, and revealed seasonal residency in the Lombok Strait for some individuals, with a particularly high affinity to the southern and western Lombok Strait. Others wandered more widely. Based on the high thermal tolerance of *M. alexandrini* revealed by the tags, *in situ* temperature and the timing of the oceanographic features in the area, the sunfish abundance on the reefs probably reflects sunfish abundance in the Lombok Strait, which in turn is likely linked to prey availability associated with the seasonal cold-water upwelling (Chapter 4).

The implications of these findings are that some, perhaps most, of the Bali sunfish subjected to diver crowding are likely to be seasonal residents of the Lombok Strait, where they seek cleaner-fish interactions at an unknown number of reef areas during the sunfish season. The effort-corrected observational data, revealing differences in the magnitude of sunfish encounter rates between sites, suggests that not all sites are equally attractive to sunfish. The extent of sunfish cleaning areas in the Lombok Strait is not known, and while it is likely to be more extensive than the known diver tourism sites, it is possible the extent of 'high quality' cleaning areas are limited. High dive pressure associated with sunfish tourism is likely to be of more concern in regard to the health and integrity of sunfish cleaning areas, than in regard to the sub-lethal impacts on individual sunfish and the wider sunfish population. Sunfish tourism management in the Nusa Penida Marine Protected Area, as well as on Bali, may benefit from focusing on limiting mechanical impact to the cleaner-fish habitats associated with the high dive pressure.

Diving and water column occupancy data from the satellite tags indicated that *M. alexandrini* in the Lombok Strait spends limited time at the surface, implying that basking behaviour is not prevalent for this species while in this area. The risk of sunfish propellar strikes associated with the rapidly increasing tourism-related boat traffic is probably limited.

The use of satellite tags with GPS capabilities were critical in my study to reveal seasonal residency in the Lombok Strait by some of the tagged Bali sunfish. The high affinity to this small strait < 70 km long and < 40 km wide, would not have been achievable through light-based geolocations. The small-scale habitat use is interesting, as the seasonal cold-water upwelling occurs over a large area along the southern coastlines of Bali, Java and other neighbouring islands. Nevertheless, the tagged sunfish did not appear to frequent these areas. The specific conditions in the Lombok Strait, with shallow thermoclines, internal waves and extensive tidally driven vertical mixing, in addition to the influence of the Indonesian Through Flow and the seasonal cold-water upwelling (Chapter 4), somehow produces an advantageous feeding location for sunfish seasonally. While the oceanographic conditions in the Lombok strait have been studied extensively, limited information is available on the ecology of the strait, at least in the international, peer-reviewed literature. The seasonal sunfish affinity to this area may point to a small, interesting ecological system for further study, and to an ecosystem potentially worthy of a wider conservation status than the current Nusa Penida Marine Protected Area.

Sea surface temperature and large scale oceanographic features have been highlighted in other studies as important in understanding sunfish movement ecology, as has the importance of smaller scale information on oceanography and productivity (e.g. Thys et al. 2015; Sousa et al. 2016b,c). My research confirms the high value of collecting fine-scale *in situ* data, in this case high resolution temperature measurements, for interpreting the seasonality of the Bali sunfish in relation to both large scale oceanographic features, i.e. the Indonesian Through Flow and the seasonal cold-water upwelling, as well as to localised tidally driven vertical mixing of the water column.

5.3 Implications of taxonomy for future sunfish research

With the renewed interest in sunfish ecology it is paramount that researchers are able to identify their study species in accordance with a taxonomy based on the latest findings, where species delineations are based on both morphology and genetics. As future changes to Molidae taxonomy are likely, it is also pertinent that researchers clearly communicate the basis for their specimen identification. Verifying the species genetically, and uploading sequences to shared platforms, such as GenBank and the Barcode of Life Data System, is of great value as it allows specimen identities to be reassessed as new information emerges on Molidae taxonomy. Furthermore, an extensive collection of sunfish sequences from around the world will greatly aid in further reviewing Molidae taxonomy.

The indication of an Atlantic and Pacific clade in *M. mola* remains intriguing. Phylogenetic analysis based on one locus in the mitochondria (D-loop) indicates genetic distances between these two clades at a putative species level. However, these clades are not evident when the analysis is based on another locus (C01) (e.g. Chapter 2, 3). The morphologies of Atlantic and Pacific *M. mola* are furthermore very similar and do not at present indicate species-level differences (Fraser-Brunner 1951; Sawai et al. 2018). However, of the three currently recognised *Mola* species, the Atlantic clade of *M. mola* is now the least researched in modern times; few genetic sequences are available on public platforms, and limited morphological data, with accompanying genetic information, are available in the published literature. In turn, the volume of historic literature on its many synonyms is vast. Perhaps somewhat ironically, *Mola* taxonomic research is now most needed where it began; in the European seas.

Taxonomic confusion is not a new phenomenon, nor is it limited to the small sunfish-twig on the tree of life. But the history of the sunfishes is a good example of the importance of taxonomy; the art of grouping and naming the natural world, tackling its fuzzy species boundaries, so that we may discuss it with each other through shared nomenclature, and determine when management of our seemingly ever increasing anthropogenic pressures is needed.

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