ICES Journal of Marine Science

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

ICES Journal of Marine Science (2015), 72(Supplement 1), i124–i127. doi:10.1093/icesjms/fsu163

Contribution to the Supplement: '*Lobsters in a Changing Climate*'

Original Article

Phyllosomata associated with large gelatinous zooplankton: hitching rides and stealing bites

Richard O'Rorke[1]*, Shane D. Lavery[1,2], Miao Wang[2], Ramón Gallego[1], Anya M. Waite[3,4], Lynnath E. Beckley[5], Peter A. Thompson[6], and Andrew G. Jeffs[2]

[1]*School of Biological Sciences, University of Auckland, Auckland, New Zealand*
[2]*Leigh Marine Laboratory, Institute of Marine Science, University of Auckland, Warkworth, New Zealand*
[3]*School of Environmental Systems Engineering and the Oceans Institute, University of Western Australia, Crawley, WA, Australia*
[4]*Alfred Wegener Institute, Helmholz Centre for Polar and Marine Research, Bremerhaven, Germany*
[5]*School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia*
[6]*Australian Commonwealth Scientific Industrial and Research Organisation, Hobart, TAS, Australia*

*Corresponding author: e-mail: roro002@aucklanduni.ac.nz

O'Rorke, R., Lavery, S. D., Wang, M., Gallego, R., Waite, A. M., Beckley, L. E., Thompson, P. A., and Jeffs, A. G. Phyllosomata associated with large gelatinous zooplankton: hitching rides and stealing bites. – ICES Journal of Marine Science, 72: i124–i127.

Received 25 June 2014; revised 24 August 2014; accepted 27 August 2014; advance access publication 3 October 2014.

During a zooplankton survey 350 km off the coast of Western Australia, we captured a large and robust zooid of a salp (*Thetys vagina*), to which six late stage larvae (phyllosomata) of the western rock lobster (*Panulirus cygnus*) were attached. High-throughput sequencing analyses of DNA extracts from midgut glands of the larvae confirmed that each phyllosoma had consumed mainly salp tissue ($\bar{x} = 64.5\% \pm 15.9$ of DNA reads). These results resolve long-standing conjecture whether spiny lobster phyllosomata attach to large gelatinous hosts to feed on them.

Keywords: food web, Indian Ocean, PCR, phyllosoma, plankton, pyrosequencing, salp, spiny lobster, Thetys, trophic level.

Introduction

For many years, there has been conjecture that spiny lobster (Family Palinuridae) phyllosoma larvae might attach themselves to large gelatinous zooplankton for assistance with locomotion and possibly to feed upon them (Jeffs, 2007). This hypothesis was partly extrapolated from *in situ* observations of phyllosomata of slipper lobsters (Family Scyllaridae; a family related to spiny lobsters) attached to, and most probably consuming, large cnidarian medusae (Shojima, 1963; Thomas, 1963; Hernkind *et al.*, 1976; Ates *et al.*, 2007) as well as captive feeding trials where the phyllosomata were capable of clinging to, and consuming the entirety of a wide variety of jellyfish medusae (Wakabayashi *et al.*, 2012). Spiny lobster phyllosomata have never been directly observed in the wild due to their small size, transparent morphology, and far offshore habitat (extending to over 1500 km offshore, Phillips *et al.*, 1979), which makes direct observations of the nature of any association with large gelatinous zooplankton unlikely. Phyllosomata can grip and manipulate other animals by the use of dactyls at the end of their limbs (Wakabayashi *et al.*, 2012), and on *Panulirus cygnus* the dactyl of the second pereiopod is particularly large and suited to this purpose (Braine *et al.*, 1979: refer video in supplementary files). However, their grip on their hosts is unlikely to survive sampling, because large gelatinous pelagic organisms, such as colonial radiolaria and siphonophores, often disintegrate when sampled in zooplankton net tows. This means that even if phyllosomata do adhere to them, it will be unlikely to observe them doing so in net hauls.

In this study, we report the capture by net tow of a large and robust zooid of a salp (*Thetys vagina*), to which six late stage phyllosomata were attached (Supplementary Figure 1). To our knowledge, this is the first record of spiny lobster phyllosomata being captured while remaining attached to another animal. To determine if the phyllosomata were feeding on the salp, high-throughput sequencing analyses were performed on DNA extracts from the midgut glands of phyllosomata carefully removed from the salp zooid. DNA methods provide a highly reliable technique for identifying the diet of pelagic predators when *in situ* observations are not possible (O'Rorke *et al.*, 2012a, b).

Table 1. Phyllosomata recovered from Thetys salp.

Phyllosoma	Size (mm)	Stage
1	27.25	9
2	18	7
3	18	7
4	16.75	7
5[a]	19.5	7
6[a]	17.5	6

Length refers to the distance from the anterior margin of the cephalic shield (cephalothorax) from between the eyestalks to the posterior tip of the pleon.
[a]DNA from these phyllosomata did not amplify.

Methods, results, and discussion

A zooplankton net tow of surface water taken on 29 August 2011 between 02:00 and 02:05 AWST at 31.09°S, 111.43°E ~350 km off the Western Australian coast following the methods of Wang *et al.* (2014) recovered a salp zoid to which six late stage *Panulirus cygnus* phyllosomata were attached (Table 1). The phyllosoma were all in a typical clinging posture adopted by phyllosoma while feeding with the ventral surface on the cephalic shield, which contains the mouthparts, pressed against the outer surface of the salp zooid. The salp was 115 × 55 mm at its longest and widest dimensions and was identified by Dr Lisa-ann Gershwin (CSIRO-Marine & Atmospheric Research, Australia) to be *Thetys vagina*, based on its unique morphology. DNA was Chelex^{TM} (Bio-Rad) extracted from tissue of the salp, and a short 18S rDNA region was PCR-amplified and sequenced using the Uni1304F and Uni1670R primers and protocol from Larsen *et al.* (2005). The sequenced *Thetys vagina* (Genbank accession KM360161) locally aligns to other salp sequences on the NCBI database. However, it does not have a perfect match on the database and was only a 91.2% match to that of the only other 18S sequence of this species previously reported on Genbank (from the NW Atlantic; Govindarajan *et al.*, 2011). From examination of the outer surface of the salp upon landing on the research vessel, it was not possible to determine whether loss of salp tissue was associated directly with predation from individual phyllosoma due to the irregularity in the surface of the salp. Therefore, a DNA approach was adopted to determine if the phyllosomata had consumed salp material. DNA was extracted from the midguts of the phyllosomata using 31 gauge syringes (O'Rorke *et al.*, 2013a) and also extracted from the codend of the net from which the salp was recovered, as well as from PCR-grade water-only negative control taken from water drained from the codend. PCR amplifications of the v7 and v9 regions of the 18S rDNA were performed according to O'Rorke *et al.*, (2012a,b) and sequenced at Macrogen (Korea) on the 454 GS platform using Titanium chemistry.

Sequenced DNA reads were processed in MOTHUR (Schloss *et al.*, 2009) using methods outlined in O'Rorke *et al.* (2013b) and it was found that DNA was successfully amplified from four of these six phyllosomata and each of these phyllosomata contained a majority of DNA fragment sequence reads that were identified as belonging to salp (Thaliacea) ($\bar{x} \pm$ SE = 64.5% ± 15.9) (Figure 1). DNA failing to amplify in approximately one-third of phyllosomata is not unusual and could be due to these two phyllosomata only recently attaching to their host, perhaps after capture in the net (O'Rorke *et al.*, 2012). However, it is very unusual to have the same OTU detected in such abundance in every phyllosoma sampled from a single site (Suzuki *et al.*, 2008; Chow *et al.*, 2010; O'Rorke *et al.*, 2012a, b; 2013b). The difference between this study and previous studies is that the phyllosomata were not randomly distributed throughout the plankton, but were all adhering to a single animal.

Two loci of the 18S rRNA gene were amplified and sequenced, which both showed the same distribution of DNA sequence reads (Figure 1), which indicates that the dominance of thaliacean reads was not an artefact of primer bias. In addition to the gut contents of the phyllosomata being sequenced, a sample of cod-end water was also collected to control for the remote possibility that we were detecting DNA that had been passively ingested by the phyllosoma. For this, 5 mL of mixed codend water was passed through a 0.5-μm syringe filter (Millipore) into 10 mL of pre-chilled EtOH and stored at −20°C. A 0.5-μm filter was used because this is the exclusion size of the filter press of late stage phyllosomata (Smith *et al.*, 2009; Simon *et al.*, 2012). Perhaps surprisingly, this control contained no salp DNA, but was dominated by DNA from colonial radiolaria. Colonial radiolaria are a significant component of the zooplankton assemblage in the East Indian Ocean, although they do disintegrate when sampled by nets (Stemmann *et al.*, 2008), and it is therefore not surprising that their DNA was detected in the codend sample.

After salp DNA, the second most abundant organisms in each phyllosoma were not identical between samples, with phyllosomata 2 and 4 containing ~25% of DNA from an anthozoan (Cerianthia) and a bony fish, respectively. This suggests that each phyllosoma was either feeding on a different prey item before encountering the salp or that they were also feeding on other food sources while attached to the salp. Captive phyllosomata have been observed to feed on a range of gelatinous zooplankton such as fish larvae, chaetognaths, ctenophores, and cnidarians (Mitchell, 1971; Kittaka, 1997), which are all a significant part of the plankton assemblage of the East Indian Ocean (Säwström *et al.*, 2014). In a previous experiment, it has been determined that *P. cygnus* phyllosomata are preferential feeders that consume arrow worms over either salps or krill in a prey-choice experimental design (Saunders *et al.*, 2012). This, along with the present study, indicates that *P. cygnus* phyllosomata are opportunistic predators, attaching themselves and feeding on large prey items, such as salps when they are available. This is consistent with the fact that salps are relatively poor in lipids, protein, and available energy (Wang and Jeffs, 2013; Wang *et al.*, 2013), but they are large and represent a high level of readily digestible biomass that is a guaranteed meal in the absence of better prey options. *P. cygnus* larvae may not consume the entirety of their hosts, unlike some slipper lobster larvae (Wakabayashi *et al.*, 2012), because it is not clear that this is a good strategy for larvae in oceanic waters with low productivity. However, *T. vagina* have considerable regenerative properties (Hirose *et al.*, 2005) and it would be valuable to learn if salp tissue can regenerate at a rate that matches consumption by phyllosomata. It has been observed that even after *T. vagina* zooids are emptied into "barrels" to become the homes of Phronimids (a Hyperiid amphipod), that the salp tissue continues to regenerate (Hirose *et al.*, 2005). Salps have very rapid growth rates, with several species increasing their body length by over 5% per hour, and some at a staggering 20% per hour (Madin and Deibel, 1998). Unfortunately, there are no data on the growth rates of *Thetys*, but they are the largest known salp (>300 mm length, Nakamura and Yount, 1958) and they can rapidly increase in numbers in response to bursts in productivity (Iguchi, 2006). Furthermore, phyllosomata that attach themselves to large animals that undergo vertical migration would be able to track the diurnal changes in prey density and therefore save considerable energy.

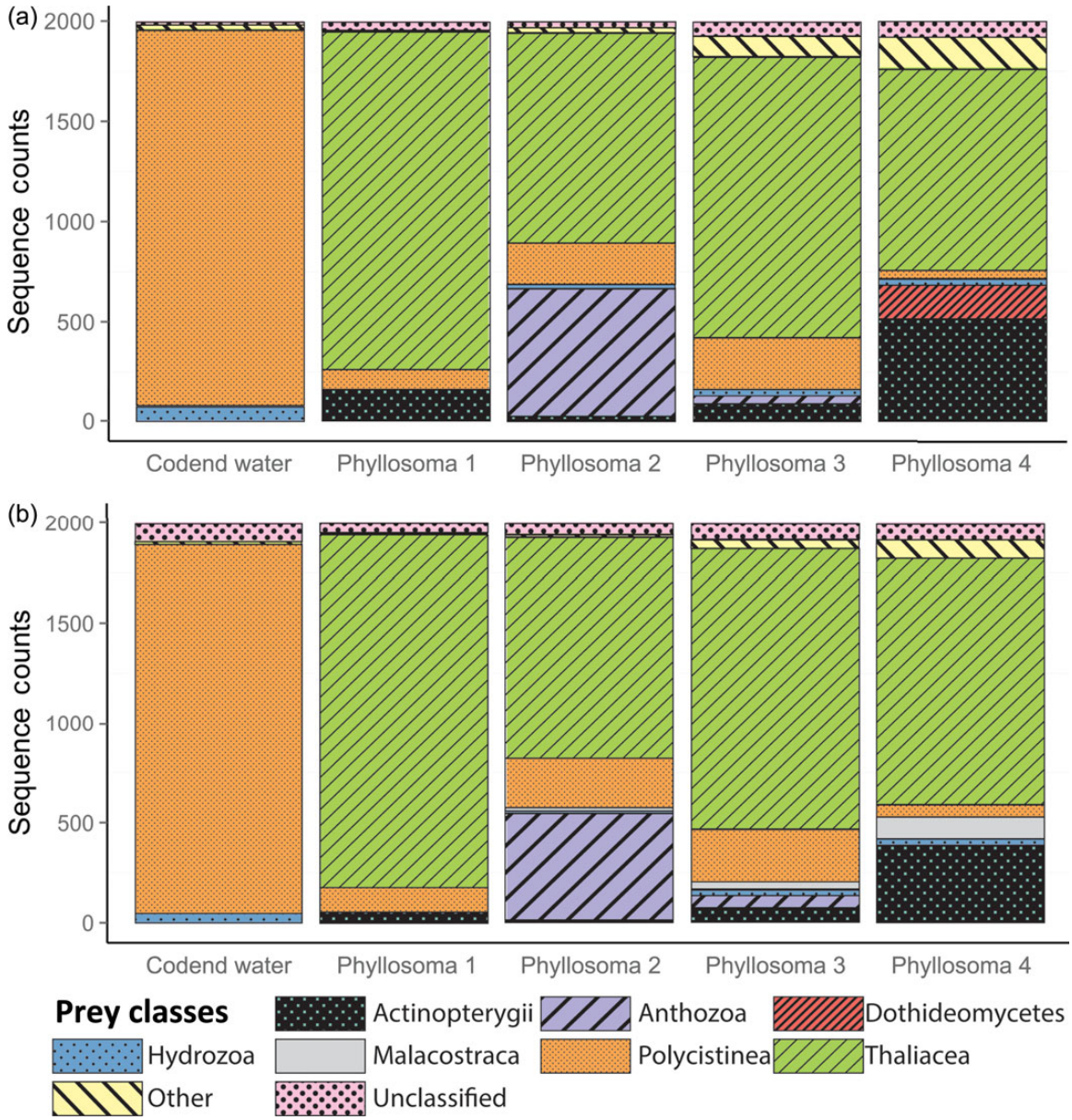

Figure 1. Distribution of reads across samples. The relative proportions of DNA sequence reads for the four phyllosomata that yielded at least 2000 reads for the (a) 18S v7 and (b) 18S v9 rRNA loci. A sample of water was also taken from the net codend to control against the possibility that phyllosomata guts contain soluble DNA ingested after capture. Both loci are concordant and show that phyllosomata contain a majority of Thaliacea (salp) DNA. In contrast, the codend water mostly contains DNA from colonial radiolarian (Polycystinea).

This is important because saving energy has considerable implications for the abilities of the spiny lobster post-larvae (pueruli) to subsequently migrate back onshore (Wilkin and Jeffs, 2011; Fitzgibbon *et al.*, 2013).

Conclusion

The unique discovery of spiny lobster phyllosomata attached and feeding on a large gelatinous zooplankter resolves conjecture that these animals share this behaviour of the larvae of their close relatives, the slipper lobsters. Sequencing of the gut contents of these spiny lobster phyllosomata confirms that they not only adhere to but also ingest the tissues of their gelatinous hosts. Locating and attaching themselves to large prey items could be an effective opportunistic feeding strategy that enables phyllosomata to survive in sparsely populated and oligotrophic oceanic waters. Importantly, by attaching to a large vertically migrating animal, the phyllosomata are able to conserve energy by not swimming, as well as having access to a meal of relatively magnificent size.

Acknowledgements

Dr Lisa-ann Gershwin (CSIRO-Marine & Atmospheric Research, Australia) for salp ID. The RV Southern Surveyor was provided by the Australian Marine National Facility under Grant SS05-2010. This project was funded by a grant from the Fisheries Research and Development Council of Australia under FRDC PROJECT NUMBER: 2010/047. The study was conducted with

the financial support of the Australian Research Council's Industrial Transformation Research Hub funding scheme (project number IH 120,100,032).

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

References

Ates, R., Lindsay, D., and Sekiguchi, H. 2007. First record of an association between a phyllosoma larva and a *Prayid* siphonophore. Plankton and Benthos Research, 2: 67–69.

Braine, S. J., Rimmer, D. W., and Phillips, B. F. 1979. An illustrated key and notes on the phyllosoma stages of the western rock lobster *Panulirus cygnus*. CSIRO Australian Division of Fisheries and Oceanography, Perth, 10 pp.

Chow, S., Suzuki, S., Matsunaga, T., Lavery, S., Jeffs, A. G., and Takeyama, H. 2010. Investigation on natural diets of larval marine animals using peptide nucleic acid-directed polymerase chain reaction clamping. Marine Biotechnology, 13: 305–313.

Fitzgibbon, Q. P., Jeffs, A. G., and Battaglene, S. C. 2013. The Achilles heel for spiny lobsters: the energetics of the non-feeding post-larval stage. Fish and Fisheries, 15: 312–326

Govindarajan, A. F., Bucklin, A., and Madin, L. P. 2011. A molecular phylogeny of the Thaliacea. Journal of Plankton Research, 33: 843–853.

Hernkind, W, Halusky, J., and Kanciruk, P 1976. A further note on phyllosoma larvae associated with medusae. Bulletin of Marine Science, 26: 110–112.

Hirose, E., Aoki, M. N., and Nishikawa, J. 2005. Still alive? Fine structure of the barrels made by *Phronima* (Crustacea: Amphipoda). Journal of the Marine Biological Association of the UK, 85: 1435–1439.

Iguchi, N. 2006. Horizontal distribution of Thetys vagina Tilesius (Tunicata, Thaliacea) in the Japan Sea during spring 2004. Journal of Plankton Research, 28: 537–541.

Jeffs, A. G. 2007. Revealing the natural diet of the phyllosoma larvae of spiny lobster. Bulletin of Fisheries Research Agency (Japan), 20: 9–13.

Kittaka, J. 1997. Culture of larval spiny lobsters: a review of work done in northern Japan. Marine and Freshwater Research, 48: 923–930.

Larsen, J., Frischer, M., Rasmussen, L., and Hansen, B. 2005. Single-step nested multiplex PCR to differentiate between various bivalve larvae. Marine Biology, 146: 1119–1129.

Madin, L. P., and Deibel, D. 1998. Feeding and energetics of Thaliacea. *In* The Biology of Pelagic Tunicates, pp. 81–103. Ed. by Q. Bone. Oxford University Press, New York.

Mitchell, J. 1971. Food preferences, feeding mechanisms, and related behavior in phyllosoma larvae of the California spiny lobster, Panulirus interruptus (Randall). San Diego State College. MSc thesis.

Nakamura, E. L., and Yount, J. 1958. An unusually large salp. Pacific Science, 12: 181.

O'Rorke, R., Jeffs, A. G., Fitzgibbon, Q. P., Chow, S., and Lavery, S. 2013a. Extracting DNA from whole organism homogenates and the risk of false positives in PCR based diet studies: a case study using spiny lobster larvae. Journal of Experimental Marine Biology and Ecology, 441: 1–6.

O'Rorke, R., Lavery, S., Chow, S., Takeyama, H., Tsai, P., Beckley, L. E., Thompson, P. A., *et al.* 2012a. Determining the diet of larvae of western Rock Lobster (*Panulirus cygnus*) using high-throughput DNA sequencing techniques. PLoS ONE, 7: 1–10.

O'Rorke, R., Lavery, S. D., Wang, M., Nodder, S. D., and Jeffs, A. G. 2013b. Determining the diet of larvae of the red rock lobster (*Jasus edwardsii*) using high-throughput DNA sequencing techniques. Marine Biology, 161: 551–563.

O'Rorke, R., Lavery, S., and Jeffs, A. 2012b. PCR enrichment techniques to identify the diet of predators. Molecular Ecology Resources, 12: 5–17.

Phillips, B. F., Brown, P., Rimmer, D., and Reid, D. 1979. Distribution and dispersal of the phyllosoma larvae of the western rock lobster, *Panulirus cygnus*, in the South-Eastern Indian Ocean. Australian Journal of Marine and Freshwater Research, 30: 773–783.

Saunders, M. I., Thompson, P. A., Jeffs, A. G., Säwström, C., Sachlikidis, N., Beckley, L. E., and Waite, A. M. 2012. Fussy feeders: phyllosoma larvae of the western rock lobster (*Panulirus cygnus*) demonstrate prey preference. PLoS ONE, 7: e36580.

Säwström, C., Beckley, L. E., Saunders, M. I., Thompson, P. A., and Waite, A. M. 2014. The zooplankton prey field for rock lobster phyllosoma larvae in relation to oceanographic features of the south-eastern Indian Ocean. Journal of Plankton Research, 36: 1003–1016.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., *et al.* 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology, 75: 7537–7541.

Shojima, Y. 1963. Scyllarid phyllosoma's habit of accompanying the jellyfish. Bulletin of the Japanese Society of Scientific Fisheries (Japan), 29: 349–353.

Simon, C. J., Carter, C. G., and Battaglene, S. C. 2012. Development and function of the filter-press in spiny lobster, *Sagmariasus verreauxi*, phyllosoma. Aquaculture, 370–371: 68–75.

Smith, G., Hall, M. W., and Salmon, M. 2009. Use of microspheres, fresh and microbound diets to ascertain dietary path, component size, and digestive gland functioning in phyllosoma of the spiny lobster *Panulirus ornatus*. New Zealand Journal of Marine and Freshwater Research, 43: 205–215.

Stemmann, L., Youngbluth, M., Robert, K., Hosia, A., Picheral, M., Paterson, H., Ibanez, F., *et al.* 2008. Global zoogeography of fragile macrozooplankton in the upper 100–1000 m inferred from the underwater video profiler. ICES Journal of Marine Science, 65: 433–442.

Suzuki, N., Hoshino, K., Murakami, K., Takeyama, H., and Chow, S. 2008. Molecular diet analysis of phyllosoma larvae of the Japanese spiny lobster *Panulirus Japonicus* (Decapoda: Crustacea). Marine Biotechnology, 10: 49–55. 7 pp.

Thomas, L. R. 1963. Phyllosoma Larvæ associated with Medusæ. Nature, 198: 208–208.

Wakabayashi, K., Sato, R., Hirai, A., Ishii, H., Akiba, T., and Tanaka, Y. 2012. Predation by the phyllosoma larva of *Ibacus novemdentatus* on various kinds of venomous jellyfish. The Biological Bulletin, 222: 1–5.

Wang, M., and Jeffs, A. G. 2013. Nutritional composition of potential zooplankton prey of spiny lobster larvae: a review. Reviews in Aquaculture, 5: 1–30.

Wang, M., O'Rorke, R., Nodder, S. D., and Jeffs, A. G. 2013. Nutritional composition of potential zooplankton prey of the spiny lobster phyllosoma (*Jasus edwardsii*). Marine and Freshwater Research, 65: 337–349.

Wang, M., O'Rorke, R., Waite, A. M., Beckley, L. E., Thompson, P. A., and Jeffs, A. G. 2014. Fatty acid profiles of phyllosoma larvae of western rock lobster (*Panulirus cygnus*) in cyclonic and anticyclonic eddies of the Leeuwin Current off Western Australia. Progress in Oceanography, 122: 153–162.

Wilkin, J., and Jeffs, A. G. 2011. Energetics of swimming to shore in the puerulus stage of a spiny lobster: can a postlarval lobster afford the cost of crossing the continental shelf? Limnology & Oceanography: Fluids & Environments, 1: 163–175.

Handling editor: Stéphane Plourde