https://doi.org/10.1071/MF20335_AC

Supplementary material

Dietary analysis and mesocosm feeding trials confirm the eastern rock lobster (*Sagmariasus verreauxi*) as a generalist predator that can avoid ingesting urchin spines during feeding

Jeremy K. Day[A,C], *Nathan A. Knott*[B], *Daniel S. Swadling*[A] *and David J. Ayre*[A]

[A]School of Earth, Atmospheric and Life Sciences, University of Wollongong, NSW 2500, Australia.

[B]NSW Department of Primary Industries, Fisheries Research, 4 Woollamia Road, Huskisson, NSW 2540, Australia.

[C]Corresponding author. Email: jkd018@uowmail.edu.au

Gut contents analysis of *Sagmariasus verreauxi*

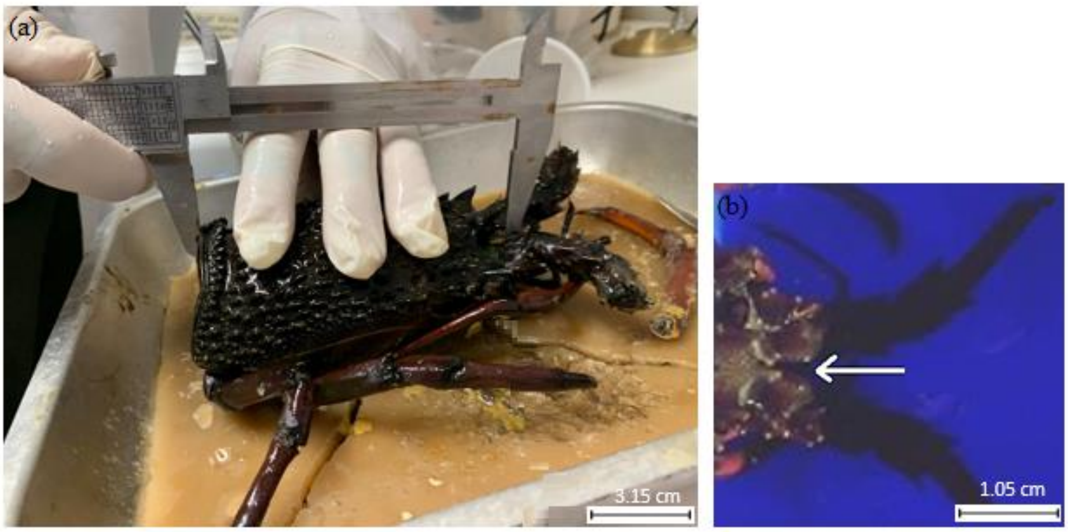

Fig. S1. We took (a) carapace measurements from lobster specimens using Vernier callipers to the nearest 0.1 mm. We measured lobsters dorsally from the posterior growth margin to (b) the middle-notch enlargement of the antennae. This measurement is more accurate for lobsters since the typically used rostrum measurement shows greater variation between individuals (Annala *et al*. 1980).

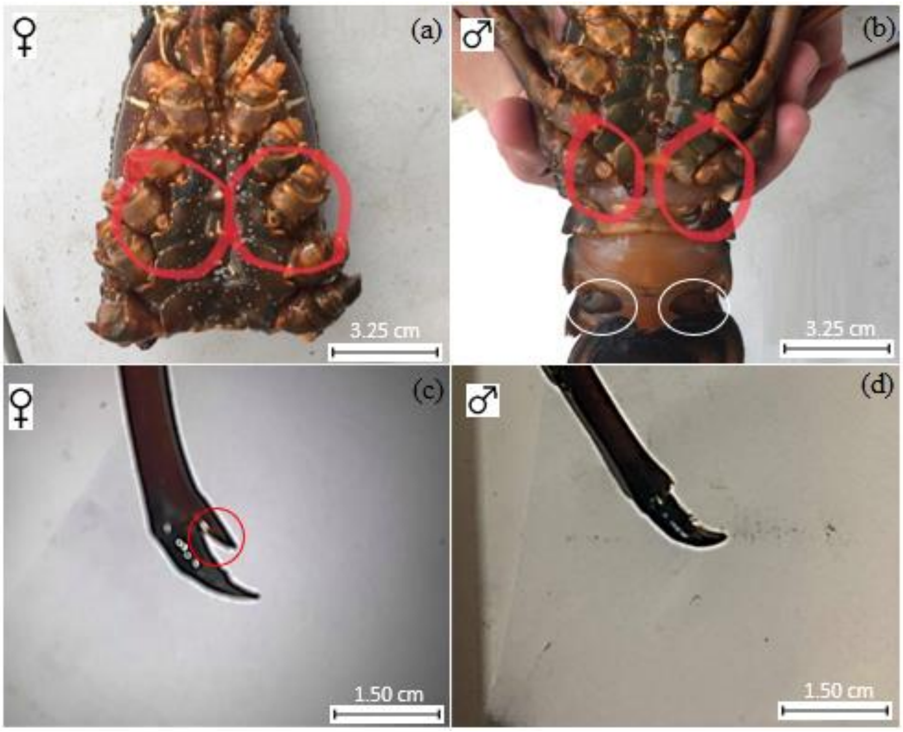

Fig. S2. Sex of lobsters is determined by observing the different gonopore arrangement between (a) Female and (b) Male individuals. However, gonopore placement can be irregular in lobsters (Linnane *et al*. 2015) so other sex characteristics should also be used. Swimmerets are (b) smaller in Males (Minagawa and Higuchi 1997) and females possess (c) a rear claw whereas (d) males do not (Schram *et al.* 2003).

Fig. S3. Dissection methodology: (a) and (b) Opening lobsters to gain access to (c) the cardiac stomach. A layer of (d) and (e) black gut integument is removed and discarded. (f) A wet stomach weight is taken, (g) the stomach is opened and flushed with water, (h) and (i) gastroliths are visible and these are removed from the emptied stomach (Haley *et al*. 2011). (j) The stomach is blotted dry and weighed. Stomach items are (k) sorted and (l) weighed. Where material was (m) sighted in the anal tract of dissected lobsters (n) this was included as gut contents material (Hyslop 1980, Williams 1981).

Fig. S4. Moult stages of dissected lobsters; (a) A pre-moult lobster showing a hard epicuticle (outer layer), a tough exocuticle (middle layer) and a soft endocuticle (base layer) (Tarsitano *et al*. 2006). (b) A soft-shelled ('post-edycsal') lobster showing a soft epicuticle, a soft exocuticle and a fragile endocuticle (Aiken 1980). (c) A post-moult lobster showing a toughened epicuticle, a soft exocuticle and a soft endocuticle. (d) A lobster showing no moult-stage; characterised by a hard epicuticle, a tough exocuticle and a soft endocuticle. In all cases the pyloric stomach (second stomach of lobsters) and hepatopancreas (liver) appear analogous to one another, and as in previous work (Thamotharan 1994) these were not distinguished during dissections.

Table S1. Taxonomic classifications of urchin parts found in the gut contents of lobsters based on spine morphometrics

We identified *C. rodgersii* and *H. erythrogramma* parts in the stomachs of lobsters based on shared and discrete characteristics of spine shape, spine hue, root hue (the base of spines), presence of dye and presence of tip coloration. Our ability to distinguish between these urchin species is confirmed by 10 blind identification trials using a dissecting microscope at 6–18 × magnification, returning a 100% success rate

Urchin species	Spine shape	Spine hue	Root hue	Dye	Tip colour
C. rodgersii	Elongate pointed, squat pointed, squat lobiform	Purple, brown, black, deep red	Brown, yellow, purple	Present	Not present
H. erythrogramma	Elongate pointed, elongate lobiform, squat pointed	Green, purple, brown, white, grey	Yellow, green, purple, white, grey	Not Present	Present

Table S2. Taxonomic classifications of (a) other hard parts and (b) non-hard parts based on morphometrics taken from the literature (Watson 1965, Lawry 1967, Novikoff and Holtzman 1970, Byrne and Hendler 1988, Rowan 1989, Wilkie 1992, Hoek *et al*. 1995, Zrzavý and Štys 1997, Tsakiris *et al*. 2004, Penney *et al*. 2007, Bownes *et al*. 2008, Clements *et al*. 2008, Witten *et al*. 2010, Thuy and Stöh 2011, Haug *et al*. 2012, Williams 2017, Ab Lah *et al*. 2019)

All observations were made using a dissecting microscope at 6–32× magnification

	Character 1	Character 2	Character 3	Character 4
(a) Other hard parts				
Mollusc	Pink/blue inner, dark outer	Few shell ‘whorls’	Fibrous hairs present	Nacre present
Gastropod	Mixed colours	Many shell ‘whorls’	Fibrous hairs absent	Nacre present
Echinoid	Urchin spine hue	Urchin spine morphology	Test ossicles present	Test pores present
Crustacean	Manipulative appendages	Locomotive appendages	$CaCO_3$ based	Na
Polychaete	Chaetae present	Cirri present	Segmented body	Na
Ophiuroid	Dorsal test plating	Ventral Ambulacral zone	Tube feet present	NA
Teleost	Fine, ray-like structure	Slightly translucent	$CaCO_3$ based	NA
(b) Non-hard parts				
Detritus	Biogenic origin	Flocculated	NA	NA
Algae	Green/red/brown pigments	Organelles present	Plant cell wall present	NA
Soft Prey	Nematode body pores	Organs present	Animal cell wall present	NA
Other	Egg structures present	Plastic Pollution present	NA	NA

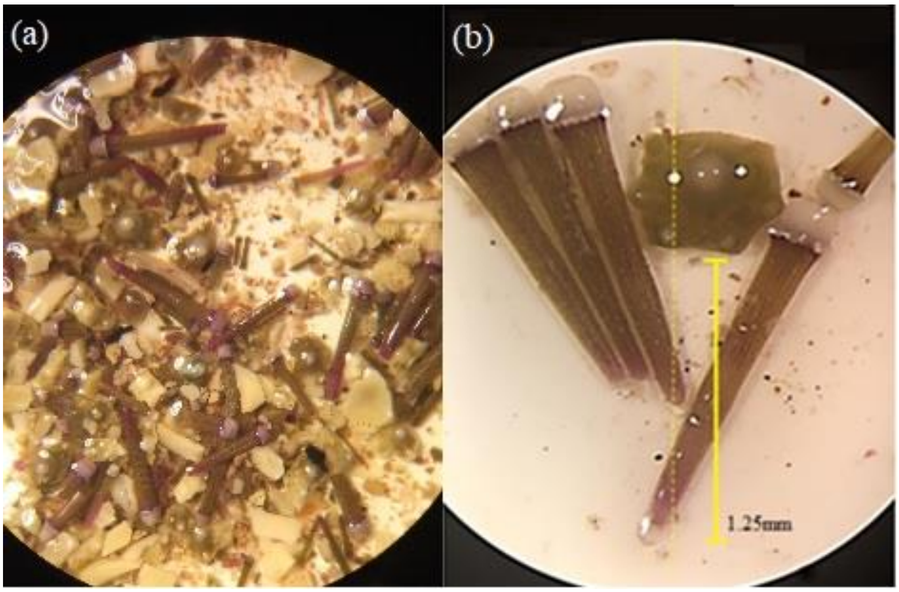

Fig. S5. Two separate detections of (a) many and (b) few urchin spines, identified to originate from *H. erythrogramma*. These examples consist of (a) urchin spines measuring ~2–3 mm and (b) tiny spines <2 mm in size. We identified urchin parts at 6–24× magnification and field of view ranged from 9.5 to 2.0 mm. Please see Fig. S6 (r) for a scale bar in (a).

Fig. S6. Urchin hard parts, showing (a) to (n) positive identifications of *H. erythrogramma* found in the gut contents across a range of fragment sizes, (o) positive identification of *C. rodgersii* spines, (p) to (r) three instances where urchin parts exclusively were found in the guts, (s) and (t) fragments of urchin test with ossicles and pores visible (Pederson and Johnson 2006; Pederson and Johnson 2008; Ebert 2013), (t) tentative ID as *H. erythrogramma*, (u) and (v) degraded test fragments with ossicles and pores visible, no ID possible (Fell 1949).

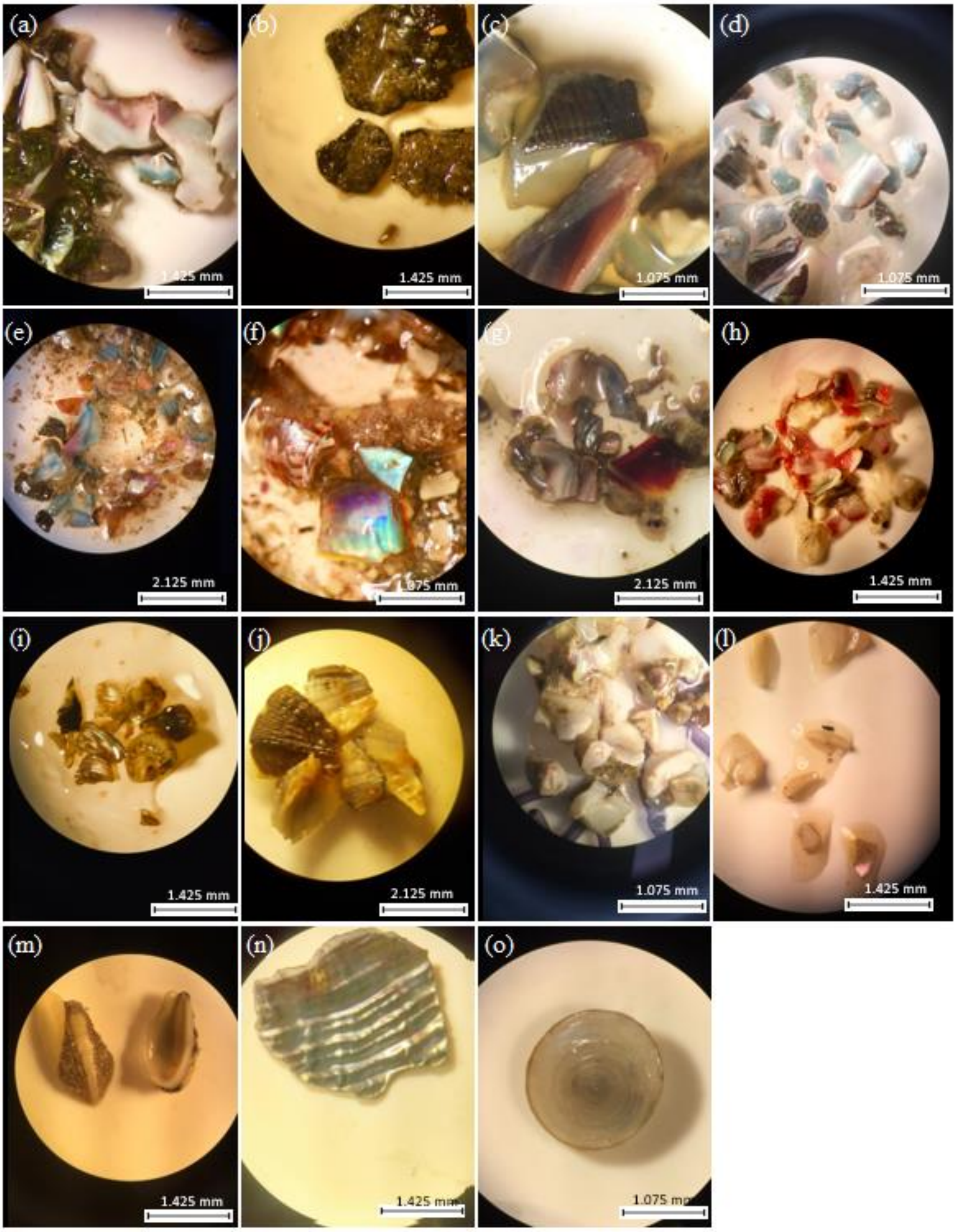

Fig. S7. Other hard parts, including (a) and (b) bivalve shell, *Mytilus* sp. (Penney *et al*. 2007; Bownes *et al*. 2008) (a) and (b), mixed bivalve and gastropod shell including *L. undulata* and *L. torquata* (Clements *et al*. 2008; Williams 2017; Ab Lah *et al*. 2019) (c) to (l), unidentified bivalve (m), an *Ostrea* sp. fragment (Hickman 1972; Arakawa 1990) (n) to (o) Unidentified gastropod.

Fig. S8. Other hard parts, including (a) to (d) decapod crustacean appendages (Haug *et al*. 2012, Zrzavý and Štys 1997), (e) and (f) appendages and (g) and (h) spines of polychaetes (Lawry 1967; Tsakiris *et al*. 2004), (i) to (k) Ophiroidean appendages and ambulacral plating of ophiuroideans (Matsumoto 1915; Wilkie 1992; Byrne *et al*. 1998; Thuy and Stöh 2011), (l) small appendage believed to have come from a small crustacean, (m) and (n) teleost fish bones (Pierce *et al*. 1991; Witten *et al*. 2010), (o) a piece of plastic fishing wire found in one lobster.

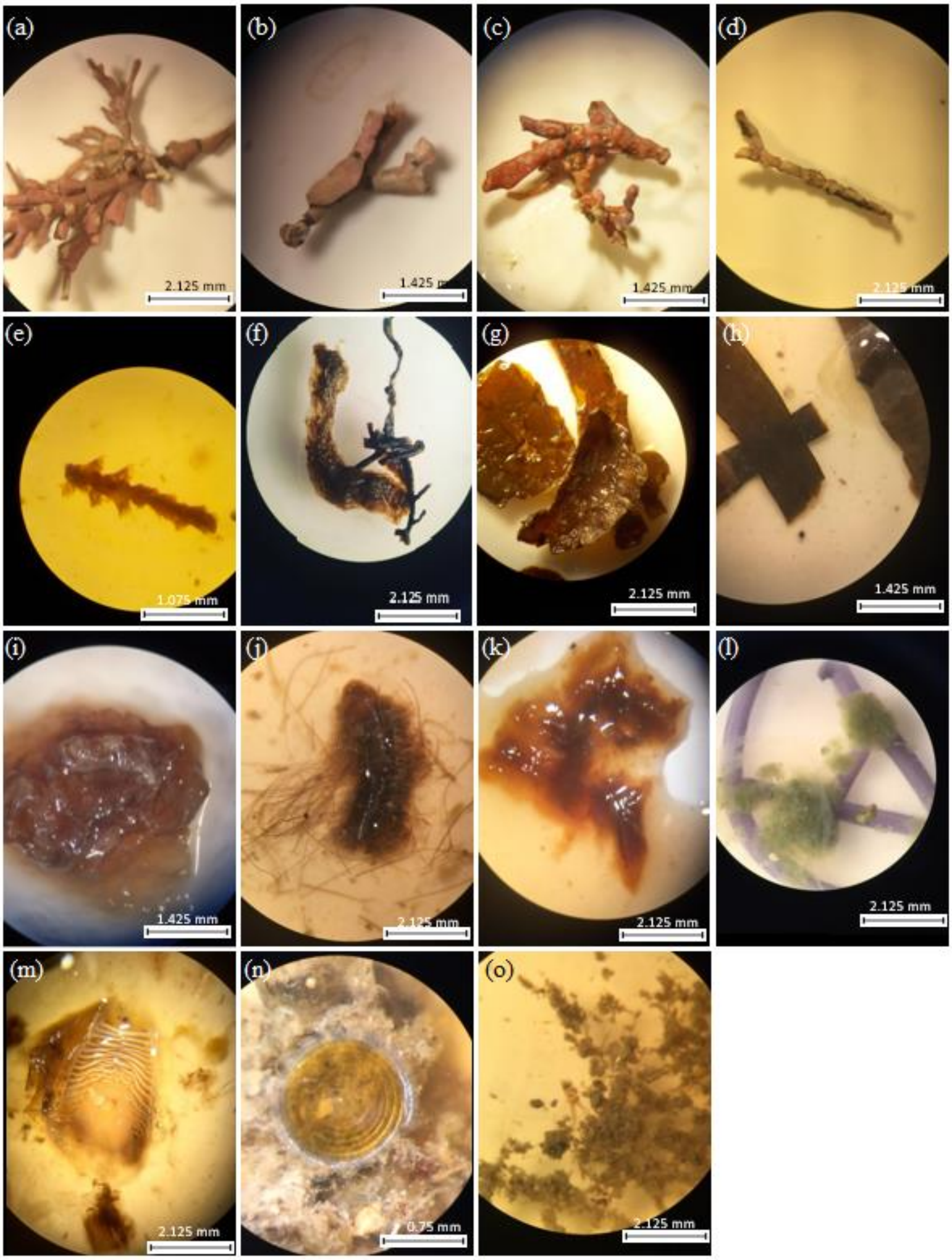

Fig. S9. Non-hard parts including various algal phyla, such as (a) to (c) calcareous red algae (Rhodophyta), (d) to (k) brown algae (Phaeophyceae) and (l) and (m) green algae (Chlorophyta) (Rowan 1989; Hoek *et al*. 1995). Detrital matter of both (n) animal and (o) plant origin (n) often co-occurred with algae (Melack 1985; O'Rorke *et al*. 2014).

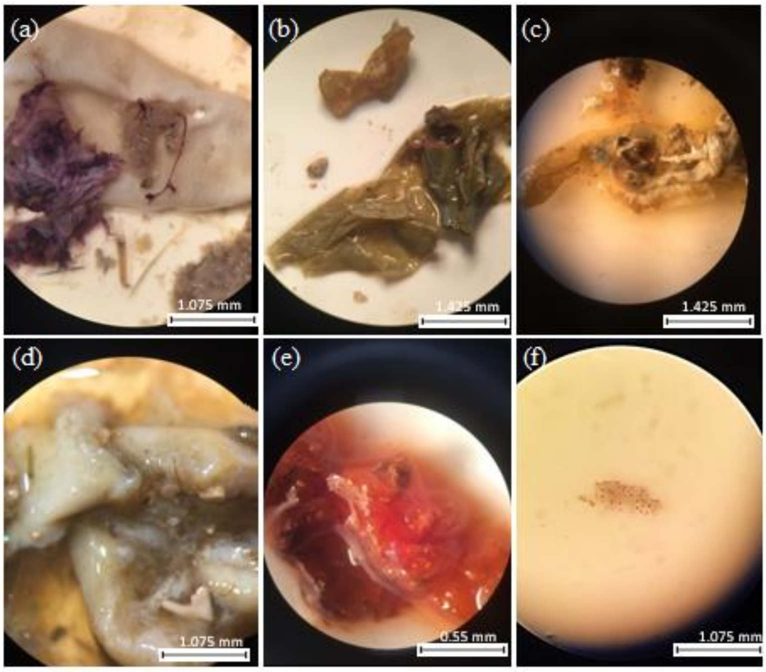

Fig. S10. Non-hard parts, including (a) soft nematode prey with gut matter and body wall pores visible (Watson 1965; Novikoff and Holtzman 1970), (b) empty nematode body wall, (c) to (d) unidentified soft matter believed to be of animal origin and (f) flocculated egg matter (Juinio and Cobb 1992; Smith *et al*. 2004).

Table S3. Model candidates examining the carapace length (Length), sex, Gut Fullness Index (GFI) and moult stage (Moult) on the probability of urchins being found within the stomach contents of lobsters using the Akaike information criterion corrected for small sample sizes (AICc)

This table shows all GLMM models with the most parsimonious model (lowest AICc and highest weight) shown first. All models include location as a random effect

Model	d.f.	AICc	ΔAICc	Weight	Marginal R^2	Conditional R^2
~Length + GFI	4	91.99	0	0.45	0.35	0.63
~Length + Sex + GFI	5	93.5	1.48	0.22	0.36	0.65
~Length + GFI + Moult	5	93.94	1.95	0.17	0.35	0.62
~Length + Sex + GFI + Moult	6	95.44	3.46	0.08	0.36	0.65
~GFI	3	96.69	4.7	0.04	0.22	0.56
~Sex + GFI	4	98.46	6.47	0.02	0.23	0.58
~GFI + Moult	4	98.72	6.73	0.02	0.22	0.56
~GFI + Sex + Moult	5	100.5	22.3	0.01	0.22	0.57

Urchin feeding trials using the eastern rock lobster (*Sagmariasus verreauxi*)

Fig. S11. Our ocean mesocosms consisted of (a) adapted recreational lobster pots covered with 2- × 2-cm plastic mesh to prevent predation by octopus and we used (b) LIFEGUARD+ brand waterproof lights and GoPro HERO3/HERO5 models attached with cable ties to observe lobster feeding. Before starting trials, we conditioned mesocosms (c) in seawater and allowed algae to accumulate on structures.

Table S4. Data table for 14 overnight feeding trials undertaken within ocean mesocosms

Carapace Length (CL, mm) denotes the size of lobsters and Test Diameter (TD, mm) denotes the size of prey urchins. Trials ran for a maximum of 14 days. Where feeding was observed the trial was terminated. Eight trials used *H. erythrogramma* whereas six trials used *C. rodgersii*

Feeding trial	CL (mm)	Duration (days)	TD (mm)	Feeding observed	Species
1	125	14	45	No	*H. erythrogramma*
			65	No	*H. erythrogramma*
2	115	14	49	No	*H. erythrogramma*
			70	No	*H. erythrogramma*
3	105	1	44	Yes	*H. erythrogramma*
			58	No	*H. erythrogramma*
4	132	1	65	Yes	*H. erythrogramma*
			45	Yes	*H. erythrogramma*
5	85	1	67	Yes	*H. erythrogramma*
			43	No	*H. erythrogramma*
6	90	11	42	Yes	*H. erythrogramma*
			65	No	*H. erythrogramma*
7	89	14	49	No	*H. erythrogramma*
			41	No	*H. erythrogramma*
8	89	14	64	No	*H. erythrogramma*
			47	No	*H. erythrogramma*
9	85	2	95	Yes	*C. rodgersi*
			40	No	*C. rodgersii*
10	90	3	98	Yes	*C. rodgersii*
			43	No	*C. rodgersii*
11	106	14	90	No	*C. rodgersii*
			29	No	*C. rodgersii*
12	111	14	108	No	*C. rodgersii*
			38	No	*C. rodgersii*
13	108	14	40	No	*C. rodgersii*
			111	No	*C. rodgersii*
14	115	14	48	No	*C. rodgersii*
			105	No	*C. rodgersii*

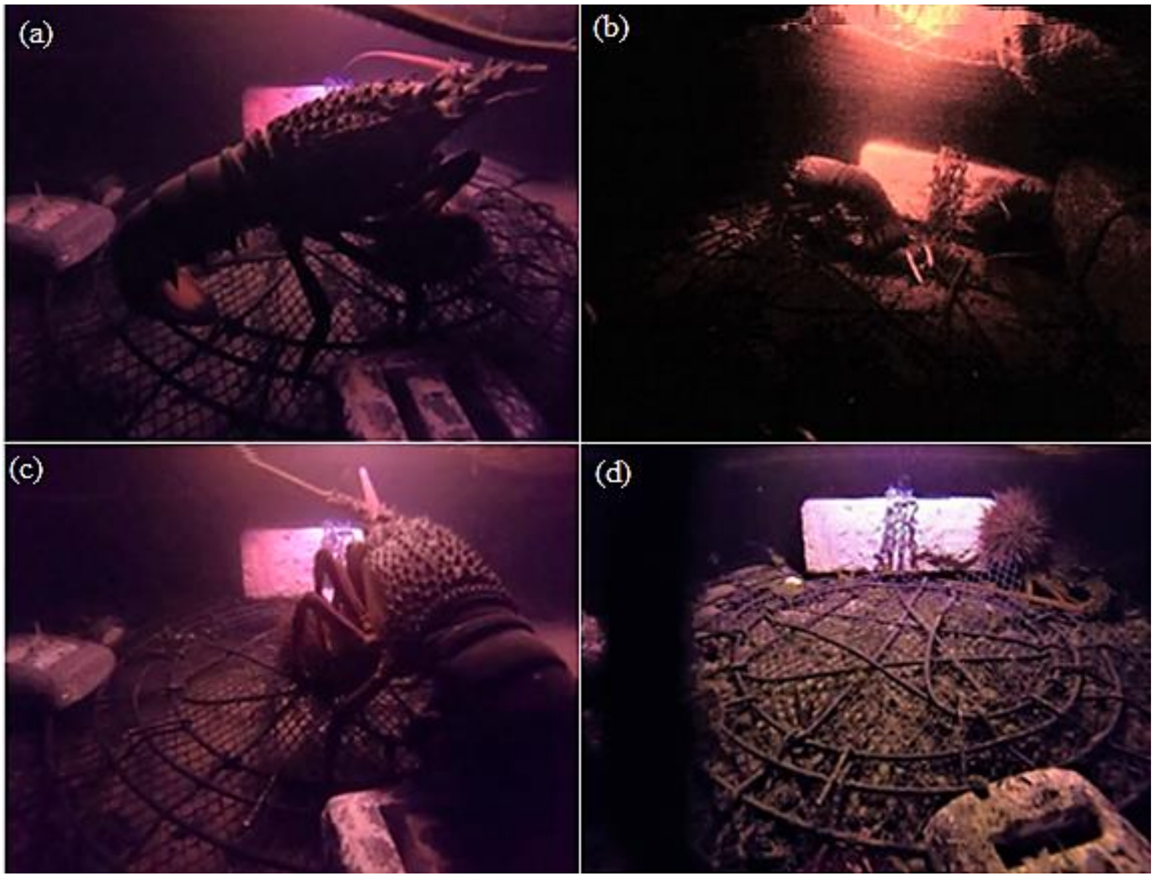

Fig. S12. Lobsters were observed to (a) eat urchins from the ventral surface, (b) use the forelegs to turn urchins over and (d) remove the feeding appendage of urchins (‘Aristotle’s lantern’) (Mayfield *et al*. 2001). Alternatively, some lobsters (b) showed no interest in the offered urchins and did not feed for the 14-day period.

References

Ab Lah, R., Bucher, D., Savins, D., Dowell, A., and Benkendorff, K. (2019). Temporal variation in condition index and meat quality of *Lunella undulata* (Turbinidae), in relation to the reproductive cycle. *Molluscan Research* **39**, 122–139. doi:10.1080/13235818.2018.1514241

Aiken, D. E. (1980). Molting and growth. In ‘The Biology and Management of Lobsters’. (Eds J. S. Cobb and B. F. Phillips.) pp. 91–163 (Academic Press: New York, NY, USA.) <b0

Annala, J. H., McKoy, J. L., Booth, J. D., and Pike, R. B. (1980). Size at the onset of sexual maturity in female *Jasus edwardsii* (Decapoda: Palinuridae) in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **14**, 217–227. doi:10.1080/00288330.1980.9515864

Arakawa, K. Y. (1990). Commercially important species of oysters in the world. *Marine and Freshwater Behaviour and Physiology* **17**, 1–13. doi:10.1080/10236249009378756

Bownes, S., Barker, N. P., and McQuaid, C. D. (2008). Morphological identification of primary settlers and post-larvae of three mussel species from the coast of South Africa. *African Journal of Marine Science* **30**, 233–240. doi:10.2989/AJMS.2008.30.2.3.553

Byrne, M. M., and Hendler, G. L. (1988). Arm structures of the ophiomyxid brittlestars (Echinodermata: Ophiuroidea: Ophiomyxidae). In ‘Echinoderm Biology: Proceedings of the Sixth International Echinoderm Conference’, August 1987, Victoria. BC, Canada, (Eds R. D. Burke, P. V. Mladenov, P. Lambert, and R. L. Parsley.) pp. 23–28 (Balkema: Rotterdam, Netherlands.)

Byrne, M., Andrew, N. L., Worthington, D. G., and Brett, P. A. (1998). Reproduction in the diadematoid sea urchin *Centrostephanus rodgersii* in contrasting habitats along the coast of New South Wales, Australia. *Marine Biology* **132**, 305–318. doi:10.1007/s002270050396

Clements, R., Liew, T. S., Vermeulen, J. J., and Schilthuizen, M. (2008). Further twists in gastropod shell evolution. *Biology Letters* **4**, 179–182. doi:10.1098/rsbl.2007.0602

Ebert, T. A. (2013). Growth and survival of post-settlement sea urchins. *Developments in Aquaculture and Fisheries Science* **38**, 83–117 . doi:10.1016/B978-0-12-396491-5.00007-1

Fell, H. B. (1949). The occurrence of Australian echinoids in New Zealand waters. *Records of the Auckland Institute and Museum* **6**, 343–346.

Haley, C. N., Blamey, L. K., Atkinson, L. J., and Branch, G. M. (2011). Dietary change of the rock lobster *Jasus lalandii* after an 'invasive' geographic shift: effects of size, density and food availability. *Estuarine, Coastal and Shelf Science* **93**, 160–170. doi:10.1016/j.ecss.2011.04.015

Haug, J. T., Maas, A., Haug, C., and Waloszek, D. (2012). Evolution of crustacean appendages. In 'The Natural History of the Crustacea'. (Eds L. Watling and M. Thiel.) pp. 34–73 (Oxford University Press: New York, NY, USA.)

Hickman, R. W. (1972). Rock lobsters feeding on oysters (note). *New Zealand Journal of Marine and Freshwater Research* **6**, 641–644.

Hoek, C., Mann, D., Jahns, H. M., and Jahns, M. (1995). 'Algae: an Introduction to Phycology', 1st edn. (Cambridge University Press: New York, NY, USA.)

Hyslop, E. (1980). Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology* **17**, 411–429. doi:10.1111/j.1095-8649.1980.tb02775.x

Juinio, M. A. R., and Cobb, J. S. (1992). Natural diet and feeding habits of the post larval lobster *Homarus americanus. Marine Ecology Progress Series* **85**, 83–91. doi:10.3354/meps085083

Lawry, J. V. (1967). Structure and function of the parapodial cirri of the polynoid polychaete, *Harmothoë. Zeitschrift für Zellforschung und Mikroskopische Anatomie* **82**, 345–361. doi:10.1007/BF00323859

Linnane, A., Pere, A., Gardner, C., and Thellier, T. (2015). Abnormal reproductive morphology in two species of spiny lobster: *Jasus edwardsii* and *Jasus paulensis. Marine Biodiversity Records* **8**, e97. doi:10.1017/S1755267215000792

Matsumoto, H. (1915). A new classification of the Ophiuroidea: with descriptions of new genera and species. *Proceedings. Academy of Natural Sciences of Philadelphia* **67**, 43–92.

Mayfield, S., De Beer, E., and Branch, G. M. (2001). Prey preference and the consumption of sea urchins and juvenile abalone by captive rock lobsters (*Jasus lalandii*). *Marine and Freshwater Research* **52**, 773–780. doi:10.1071/MF00067

Melack, J. M. (1985). Interactions of detrital particulates and plankton. In 'Perspectives in Southern Hemisphere Limnology'. pp. 209–220 (Springer: Dordrecht, Netherlands.)

Minagawa, M., and Higuchi, S. (1997). Analysis of size, gonadal maturation, and functional maturity in the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). *Journal of Crustacean Biology* **17**, 70–80. doi:10.2307/1549464

Novikoff, A. B., and Holtzman, E. (1970). Cell organelles. In 'Cells and Organelles', 2nd edn. pp. 105–106 (Holt, Rinehart and Winston: New York, NY, USA.)

O'Rorke, R., Lavery, S. D., Wang, M., Nodder, S. D., and Jeffs, A. G. (2014). Determining the diet of larvae of the red rock lobster (*Jasus edwardsii*) using high-throughput DNA sequencing techniques. *Marine Biology* **161**, 551–563. doi:10.1007/s00227-013-2357-7

Pederson, H. G., and Johnson, C. R. (2006). Predation of the sea urchin *Heliocidaris erythrogramma* by rock lobsters (*Jasus edwardsii*) in no-take marine reserves. *Journal of Experimental Marine Biology and Ecology* **336**, 120–134. doi:10.1016/j.jembe.2006.04.010

Pederson, H. G., and Johnson, C. R. (2008). Growth and age structure of sea urchins (*Heliocidaris erythrogramma*) in complex barrens and native macroalgal beds in eastern Tasmania. *ICES Journal of Marine Science* **65**, 1–11. doi:10.1093/icesjms/fsm168

Penney, R. W., Hart, M. J., and Templeman, N. D. (2007). Shell strength and appearance in cultured blue mussels *Mytilus edulis*, *M. trossulus*, and *M. edulis* × *M. trossulus* hybrids. *North American Journal of Aquaculture* **69**, 281–295. doi:10.1577/A06-044.1

Pierce, G. J., Boyle, P. R., and Diack, J. S. W. (1991). Identification of fish otoliths and bones in faeces and digestive tracts of seals. *Journal of Zoology* **224**, 320–328. doi:10.1111/j.1469-7998.1991.tb04810.x

Rowan, K. S. (1989). 'Photosynthetic Pigments of Algae.' (Cambridge University Press: Cambridge, UK.)

Schram, F. R., Ahyong, S. T., and Dixon, C. J. (2003). A new hypothesis of decapod phylogeny. *Crustaceana* **76**, 935–975 . doi:10.1163/156854003771997846

Smith, G. G., Ritar, A. J., Johnston, D., and Dunstan, G. A. (2004). Influence of diet on broodstock lipid and fatty acid composition and larval competency in the spiny lobster, *Jasus edwardsii*. *Aquaculture* **233**, 451–475. doi:10.1016/j.aquaculture.2003.11.009

Tarsitano, S. F., Lavalli, K. L., Horne, F., and Spanier, E. (2006). The constructional properties of the exoskeleton of homarid, palinurid, and scyllarid lobsters. In 'Issues of Decapod Crustacean Biology'. pp. 9–20. (Springer: Dordrecht, Netherlands.)

Thamotharan, M. (1994). Comparative physiology of dipeptide transport in lower vertebrates (fishes) and invertebrates (lobster) Ph.D. dissertation, University of Hawaii, Manoa, HI, USA.

Thuy, B., and Stöh, S. (2011). Lateral arm plate morphology in brittle stars (Echinodermata: Ophiuroidea): new perspectives for ophiuroid micropalaeontology and classification. *Zootaxa* **3013**, 1–47. doi:10.11646/zootaxa.3013.1.1

Tsakiris, D. P., Menciassi, A., Sfakiotakis, M., La Spina, G., and Dario, P. (2004). Undulatory locomotion of polychaete annelids: mechanics, neural control and robotic prototypes. In 'Annual Computational Neuroscience Meeting', 18–22 July 2004, Baltimore, MD, USA. pp. 1–3. (Foundation for Research and Technology.)

Watson, B. D. (1965). The fine structure of the body-wall in a free-living nematode, *Euchromadora vulgaris. Journal of Cell Science* **3**, 75–81.

Wilkie, I. C. (1992). Variable tensility of the oral arm plate ligaments of the brittlestar *Ophiura ophiura* (Echinodermata: Ophiuroidea). *Journal of Zoology* **228**, 5–26. doi:10.1111/j.1469-7998.1992.tb04429.x

Williams, M. J. (1981). Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). *Journal of Experimental Marine Biology and Ecology* **52**, 103–113. doi:10.1016/0022-0981(81)90174-X

Williams, S. T. (2017). Molluscan shell colour. *Biological Reviews of the Cambridge Philosophical Society* **92**, 1039–1058. doi:10.1111/brv.12268

Witten, P. E., Huysseune, A., and Hall, B. K. (2010). A practical approach for the identification of the many cartilaginous tissues in teleost fish. *Journal of Applied Ichthyology* **26**, 257–262. doi:10.1111/j.1439-0426.2010.01416.x

Zrzavý, J., and Štys, P. (1997). The basic body plan of arthropods: insights from evolutionary morphology and developmental biology. *Journal of Evolutionary Biology* **10**, 353–367. doi:10.1007/s000360050029