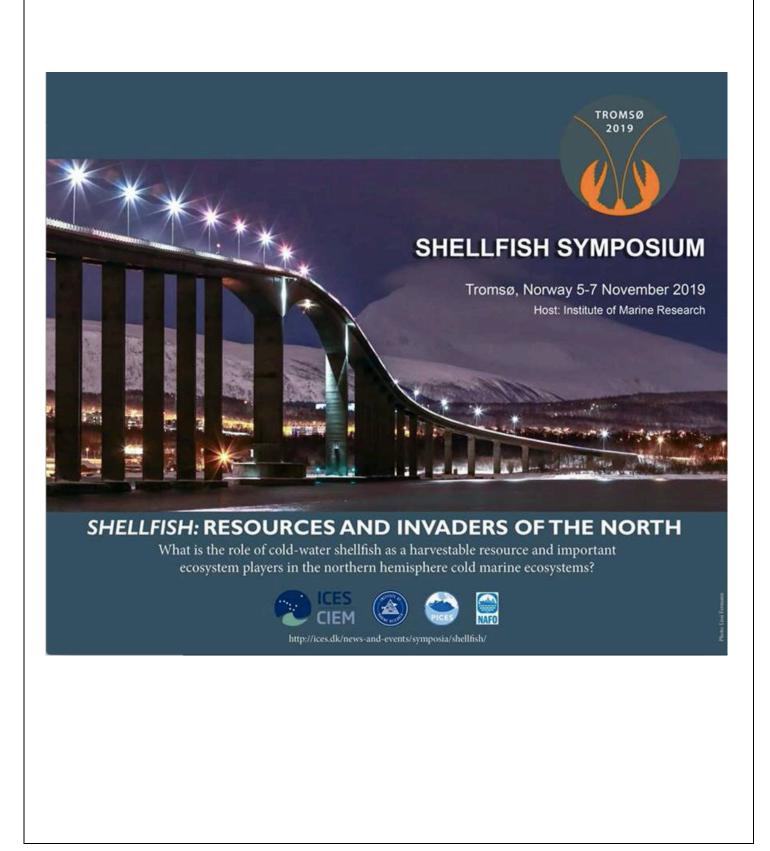


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Selected presentations will be livestreamed. Visit the conference website for all information including: registrations; the conference programme; and instructions on accessing the livestreaming facility.

www.lobsterconference2019.co.nz

Malcolm Lawson Chief Executive Officer CRA8 Rock Lobster Industry Association Inc. Join us for the biggest rock lobster event of 2019





The Department of Primary Industry and Regional Development (DPIRD) and the Western Rock Lobster (WRL) council are pleased to be hosting the 12<sup>th</sup> ICWL workshop on **18-23 October 2020** at the Esplanade Hotel in Fremantle, Western Australia. Fremantle is a major port for the western rock lobster fleet, 20 km from Perth, the capital city of Western Australia (WA). A website has been developed (https://icwl2020.com.au/) so please register your interest so we can keep you updated on the workshop.

The ICWL workshop is the week following the **World Fisheries Congress** on 11-15 October 2020 in Adelaide, South Australia (<u>wfc2020.com.au</u>) which provides an excellent opportunity to attend two world-class events during your visit to Australia. When you are planning your trip downunder you may wish to include visiting some of the numerous attractions we have to offer in Western Australia in your itinerary. We will be providing some suggestions on the website.

Planning for the conference is underway with a conference organiser, a steering committee, a local technical committee and an international advisory committee in place to help plan for the workshop. This lobster workshop returns to where it all began in Western Australia over 40 years ago to find common themes amongst the different lobster species. In recent years participation has risen to 150-200 people from 20 countries.

The theme for the 2020 workshop adopted is '**Ecosystem-based Fisheries Management (EBFM)**' as this generally represents best practice for fisheries management and reflects that fisheries research and management focus is now broader than just sustainability. Therefore we will be welcoming the presentations that focus on the biology of the lobster species and stock assessment and are also hoping to attract presentations that examine other aspects of EBFM such ecosystem effects of fishing, economic assessments, social issues, governance and compliance with management regulations. We are also hopeful of attracting papers on lobster aquaculture as this has been an important developing industry in Asia. An industry day is also planned to be an important component of the program so we are looking forward to strong support from lobster industry participants around the world.

The local technical committee has also had some strong support to include crab research papers as part of this conference as they have many similar issues to the lobsters. You may be aware that the 2<sup>nd</sup> lobster conference in St Andrews in 1985 also included a number of crab presentations. We therefore encourage presentations based on crab research at the conference.

The WRL and DPIRD are looking forward to hosting scientists, managers and industry participants in Western Australia in 2020. The steering committee and technical committee welcome any ideas that you may have for the lobster workshop such as particular sessions and special workshops that could be held as part of the ICWL12. The list of sessions that have been proposed based on past conferences and taking into account the EBFM theme of this conference are listed below. Co-hosts of the workshop Nic Sofoulis

Nick Caputi

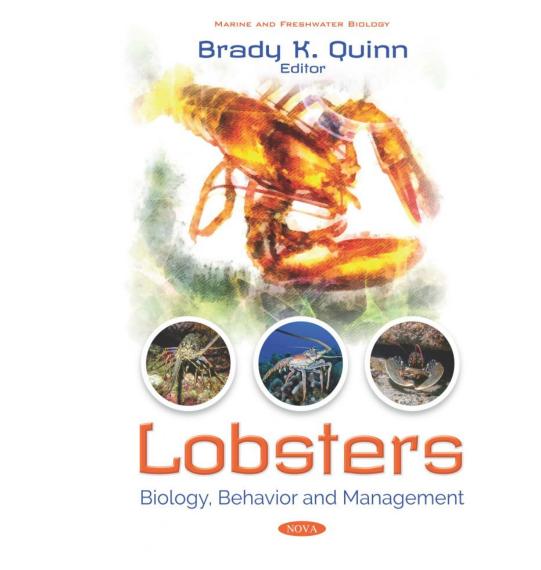
DPIRD (nick.caputi@dpird.wa.gov.au)

WRL (sofs1@bigpond.com)

### Proposed session topics for 12th ICWL – other suggestions welcome

- 1. Behaviour and Behavioural Ecology
- 2. Physiology and Neurobiology
- 3. Reproductive Biology
- 4. Diseases and Parasites
- 5. Recruitment processes
- 6. Fisheries Science and Stock Assessment
- 7. Population Dynamics, genetics and connectivity
- 8. Aquaculture
- 9. Climate Change and Oceanic Processes
- 10. Trap Design, Ghost Fishing and Gear Conflicts
- 11. Ecosystem Based Fisheries Management (EBFM)
- 12. Economic Assessments
- 13. Compliance
- 14. Recreational Fishing
- 15. Ecosystem Effects of Fishing
- 16. Marine Stewardship Council & Third Party Certification
- 17. Social Issues
- 18. Invasive species
- 19. Habitat / Dietary preferences
- 20. Anthropogenic impacts e.g. Seismic surveys
- 21. Industry Day: Industry Issues
- 22. Industry Day: Management
- 23. Resource sharing





### Lobsters: Biology, Behavior and Management Edited by Brady K. Quinn

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# The regulation of the Maine lobster fishery to protect the endangered North Atlantic right whale

### From: Erin Summers

The decline of the North Atlantic right whale, *Eubalaena glacialis* (Fig. 1), has set up a conflict in the US lobster fishery. There are two perceived sides to the issue, those who want to make sweeping changes to the US American lobster fishery and those who execute that fishery. But, as in most conflicts, there is more gray to the issue than black or white, and the plight of the right whale is one that is plagued by a severe lack of information to guide the dialogue.

After years of growth and robust calving, the right whale population began to decline from its peak in 2010 to its current status of 411 individuals (Fig. 2; Pettis et al. 2018; Pace et al. 2017). This prompted the initiation of US action in 2017 under the Marine Mammal Protection Act, action which was accelerated later that same year when 17 right whales were found dead between the Gulf of St. Lawrence in Canada and along the East Coast of the US (Unusual Mortality Event). This loss is equivalent to roughly 4% of the population. Human-caused serious injury and mortality in this species is made up of ship strikes and entanglements in fixed fishing gear, such as trap fisheries and gillnets (Knowlton and Kraus 2001). In Maine, the largest fixed gear fishery is the lobster fishery.



Figure 1. The North Atlantic right whale. Source: <u>https://www.fisheries.noaa.gov/species/north-atlantic-right-whale</u>

Maine has been engaged in the right whale issue since the formation of the Atlantic Large Whale Take Reduction Team (TRT) in 1997. It maintains seats on the TRT, including five from the lobster fishing State's industry and from the one Department of Marine Resources. This team has helped to develop a range of previous regulations in the lobster fishery, and other US Atlantic coastal fisheries, aimed at reducing the risk of entanglement for right whales. These measures include sinking groundline, trawl length minimums, weak links, and keeping floating rope off the surface.

Currently, there is turmoil in the Maine lobster fishery over the need for additional protections and how far they should go. It is a question of how much of the entanglement risk can be justifiably assigned to the Maine lobster fishery. It is a complicated issue made more difficult by the limited amount of data available on entanglements.

The dataset of entangling fishing gear is one of the smallest datasets that NOAA's National Marine Fisheries Service (NMFS) maintains. In very few cases is gear retrieved from entangled whales, and in even fewer cases can an entanglement be attributed to a specific a fishery or location. Additionally, right whales have been changing their habitat use and movements in search of changing supplies of their primary prey, the copepod, Calanus finmarchicus (Record et al. 2019). In the winter and early spring fewer have been observed in the Gulf of Maine but more in Cape Cod Bay (Davis et al. 2018). They have decreased their use of the Bay of Fundy, a once heavily used late summerearly fall feeding habitat (Davies et al. 2019), but are traveling to the Gulf of St. Lawrence in the summer to feed. This has prompted the Canadian government to implement new regulations for their fixed gear and shipping industries (Canadian Science Advisory Report 2019/028). However, uncertaintly around when and where right whales become entangled has hindered progress on implementing conservation measures that have tangible results.

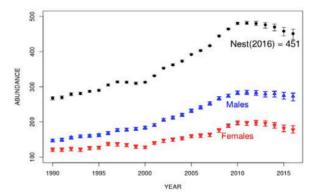


Figure 2. Abundance estimate for North Atlantic right whales. Estimates are the median values of a posterior distribution from modeled capture histories. Also shown are sex-specific abundance estimates.

The US and Canadian governments are both working on this issue and holding bi-lateral talks to exchange information. The US laws, the Marine Mammal Protection Act and the Endangered Species Act, have specific and strict goals that are the call to action to increase regulations on fixed fishing gear industries, in this case, the American lobster fishery. But successful protections for right whales in US waters must be met with equivalent measures in Canadian waters.

The Maine lobster fishery has a longstanding commitment to conservation and stewardship of the resource. Regulations should be developed in a way that not only consider the safety and efficiency of the industry, but also achieve true benefits for the right whale population.

How to move forward? On a hot July weekend I found myself standing in a garage with a group of fishermen and scientists working together to brainstorm and test gear modifications to address the right whale entanglement issue. Data from this work will be provided to NMFS as a baseline for discussions around using weak points on vertical lines to reduce the risk that entanglements will result in serious injury or a mortality. This kind of collaboration, where science and industry work together proactively to develop solutions, illustrates Maine's continued commitment to informed decision making. The State of Maine and the Maine lobster fishery will continue to be engaged partners in the process of determining new management measures that are the right fit for both the protection of right whales and the continuation of this important fishery.

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# Fishers helping whales: Co-existing in the Gulf of St. Lawrence

From: Lyne Morissette, Ph.D.

Atlantic Canada's Gulf of St. Lawrence supports a vast and rich ecosystem, providing nutrients, shelter and food to a variety of species, from plankton to marine mammals, and supporting a prominent fishery for species such as herring, cod, shrimp, crab, or lobster.

About a dozen species of cetaceans also occur in the Gulf, from small species such as harbor porpoises to the largest animal on the planet, the blue whale. Less frequent until the beginning of this decade, the North Atlantic right whale (NARW), an endangered species with a total population of about 400 individuals left, is now more and more present in the Gulf. At first, it was thought that we just had "more eyes at sea" to observe them in an area that was known to be an "occasional range of distribution" (its main range being the Gulf of Maine and the Bay of Fundy), but it is now evident that they increasingly use the northern part of their distribution range as a potential feeding habitat. One hypothesis that could explain this is that they might follow their preferred food, zooplankton copepods of the Calanus species, which is also found in the Gulf of St. Lawrence, in increasing increased concentrations. The energy required to swim long distances to find food might suggest that the whales are not getting sufficient nutrition, and arrive in the Gulf in poor health.

Occupying this new territory leads to new interactions with other users of the marine environment, and thus new conservation challenges in this area. Following an important mortality event in 2017 (17 dead NARW; 15 in Canadian waters, due to different mortality causes including marine shipping, entanglements in fishing gear, and others), different management measures were enforced on marine shipping, as well as for crab and lobster fishermen in 2018, in an urgent attempt to reduce risks of mortality on this species in the Gulf of St. Lawrence.

These radical measures had an important, unfortunately unconsidered, although impact on fishing communities. Willing to increase the efficiency to "co-exist" in the Gulf of St. Lawrence and inspired by the long-time efforts developed by their US neighbours, lobster and crab fishers decided to work in collaboration with scientists, the governments, NGOs and different institutions to protect the North Atlantic right whales in their region.

Coexisting in the Gulf relies on a two-step approach: first, the overlap between whales and fishing gear must be reduced. This means acquiring up-to-date information on when and where the whales are in the Gulf, and implementing management policies and regulations for fisheries to occur at times or in areas where whales are not there, therefore reducing the risks a whale encounters fishing gear. In order for it to must this be an inclusive work. management/mitigation system, where fishermen are involved in early stages of the process. Their understanding of their operation, the observations they make about whale presence in the Gulf, and their knowledge of the area are precious sources

of information that can make the management even more efficient. Involving the industry in early steps of the process, including finer scale data collection and knowledge improving of whales' spatiotemporal distribution in fishing areas, also helps compliance on management measures.

The second step of the approach is to develop new technologies of fishing gear to reduce entanglements or fatalities when fishermen and whales are occurring on the same area and at the same time. Since 2018, tremendous efforts were made by Canadian fishing associations to involve, develop and test different technological solutions for fishing gear to be less of a risk for whale entanglement or injuries. One project amongst them was developed by the Acadian Crabbers Association, in collaboration with M-Expertise Marine, Corbo Engineering, the Government of New Brunswick, and the Atlantic Fisheries Fund of Fisheries and Oceans Canada. Proposed solutions include ropeless traps, ropes with breaking-strength, electronic reduced monitoring for real time transmission of data whale related to sightings and entanglements, regulatory monitoring activities, electronic logbooks, new coding technologies for traceability of fishing gear, rehabilitation of the natural and infrastructure of the seabed through ghost gear retrieval. Here again, the fishermen's knowledge is crucial in every step of the process, from conception to sea trials. After witnessing 2 years of research and development, I can testify that a lot of efficiency was gained in every developed technology by involving the industry and by counting on fishermen's knowledge of the gear or the way they use it at sea.

My field, conservation biology, is a multidisciplinary science that has developed to address the loss of biodiversity, around two central goals: evaluating human developing practical impacts and approaches to prevent the extinction of species. With a species facing extinction, such as the North Atlantic right whale, we do not have the luxury of being without knowledge, ideas, or people on the field. whales are ambassadors Right of collaboration, and one of their greatest legacy is hope.

Of course, nobody would have wished for the 2017 hecatomb to happen. However, this unprecedented an wave of created cooperation in Atlantic Canada. And throughout this crisis we face, there is place for hope: this level of collaboration between different fisheries (crab, lobster, coastal or offshore) and within them (through different associations) has never been seen before. Everybody seems to care about whales in the gulf, and work hand-in-hand to make sure both the whales and the fisheries survive. That's what coexisting is all about in the end: working together to find the most efficient conservation solutions for the North Atlantic right whale, at a time where co-operation and kindness (to others and to nature) are the key to make a significant difference, for a better future.

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# Gene regulatory response in *Homarus americanus* to endcentury oceanic conditions

### From: Maura Niemisto

The northwest Atlantic is expected to be hard-hit by the projected changes in our world's oceans over the next century, putting its marine resources at great risk. On one hand, this region has experienced a faster rate of warming than 99% of the world's oceans over the last thirty years (Balch et al., 2012; Pershing et al., 2015). In addition, the coastline of the Northeastern United States and Atlantic Canada is susceptible to higher rates of acidification, due to the depressed buffering capacity contributed by elevated freshwater inputs from coastal rivers and melting sea ice entering the Labrador current (Salisbury et al., 2008; Fabry et al., 2009; Gledhill et al., 2015). In fact, moderate projections expect the Gulf of Maine to drop 0.2 pH units and increase 3°C by the year 2100 (IPCC, 2013).

This scale of change has implications for the iconic American lobster Homarus americanus fishery, which holds the title for the most valuable single-species fishery in North America (NOAA, 2019; DFO, 2019). Already, the lobster has demonstrated a response to warming temperatures, as its center of distribution has shifted northward, with fisheries at the southern end of the range collapsing from stressful summer conditions and rising prevalence of epizootic shell disease (LeBris et al., 2018). Warming temperatures have also been implicated in the downward shift of size of maturity of the species (LeBris et al., 2016; Haar et al., 2017; Waller et al., 2019). However, the effects of decreasing pH have yet to be recognized as an immediate concern, since adult lobsters can manipulate their intracellular pH levels under current conditions (Whiteley, 2011; Gledhill *et al.*, 2015).

Regardless, the recent changes in distribution. well end-century as as projections have scientists concerned about the resilience of the species, particularly along the southern extent of its range. Early life stages may be especially vulnerable to warming and acidification, since they occupy the upper water column where rapid temperature and pH shifts are commonplace (Kurihara et al., 2007; Pörtner & Farrell, 2008). Furthermore, larvae undergo rapid, physiologically demanding transitions between stages, associated with high mortality. Not enough is understood about how changing oceanic conditions might restrict biological bottlenecks within these early life stages and create further strain on recruitment down the line.

To date, six studies have evaluated larval and postlarval physiological responses to sea water acidification (Keppel et al., 2012; Agnalt et al., 2013; Small et al., 2015; Waller et al., 2017; Rato et al., 2017). Only three of those have included a temperature component as a joint-stressor, and these studies have repeatedly identified temperature to be a stronger driver of physiological and biological response than end-century levels of CO<sub>2</sub> (Agnalt et al., 2013; Small et al., 2015; Waller et al., 2017). Those that have identified a CO2 effect have often been ambiguous, or only report responses at extreme levels much higher than those predicted by the end of the century (Agnalt et al., 2013; Small et al., 2015; Waller et al., 2017; Menu-Courey, 2019). These data may indicate that early life stage lobster, like their

adult counterparts, do not respond much to acidification within the range of these future projections. Alternatively, there could be compensatory mechanisms on the molecular scale that have not yet been detected in past studies (Waller *et al.*, 2017).

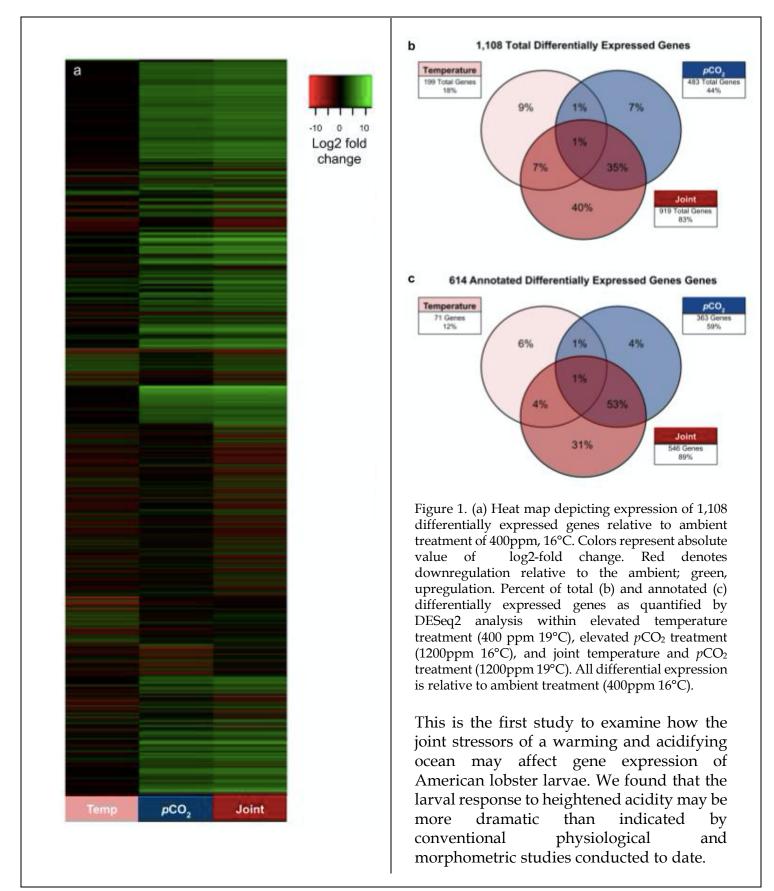
Our study was the first to measure gene regulatory response to both elevated temperature and  $pCO_2$  as single and joint stressors, as a means to detect cellular-level response. We collected newly hatched larvae from ovigerous females from southern New England, USA, and reared them in a full factorial design in ambient and end-century temperature and  $pCO_2$  of the Gulf of Maine. We collected stage IV postlarvae for RNA extraction and employed high-throughput to assess the sequencing differential expression of genes when reared under our conditions DESeq2 treatment using software.

Between our four laboratory treatments (ambient  $pCO_2$  and temperature, elevated  $pCO_2$  only, elevated temperature only, and elevated CO<sub>2</sub> and temperature) we observed 1,108 differentially expressed genes relative to the ambient conditions. Unlike past joint stressor experiments with lobster larvae, which have consistently identified temperature as the dominant stressor, we found higher gene regulatory response to occur when CO<sub>2</sub> was introduced as a stressor. In addition, we found that the joint treatment of elevated temperature and CO<sub>2</sub> had a compounding effect, resulting in 83% of the 1,108 differentially expressed genes reacting, compared to 18% when only temperature was increased, and 44% with only CO<sub>2</sub> increased (Figure 1).

Further, based on a library of sequenced genes of known function, we were able to

identify 55% of the differentially expressed genes. This software-assisted process, called annotation, identified specific "genes of interest" tied to cuticle development and immune function. We found that under higher temperatures postlarvae downregulated important genes related to cuticle formation and calcification, but showed little response in genes related to immune response. Elevated  $pCO_2$ , however, resulted in upregulation of cuticle binding molecules, chitinase (used to break down chitin), and a suite of immune and stress response genes such as HSP70, hemocyanin, mannose-binding protein, crustin, c-type lectin, and glutathione S-transferase. Finally, the two stressors together resulted in a combination of regulation seen in singlestressors, but with more genes differentially regulated under these same categories (Figure 2).

These results reveal an interactive effect of end-century-predicted  $pCO_2$ and temperature on genes controlling the exoskeleton formation immune and functioning Homarus of postlarval americanus. They contrast with previous studies reporting physiological responses to warming to be greater than to elevated CO<sub>2</sub> (Small et al., 2015; Waller et al., 2017). It is crustaceans have molecular possible mechanisms that compensate for these physiological stressors to maintain homeostasis, and therefore manifest little or outward response measured no physiological or morphometric endpoints, but are better revealed with gene expression results highlight analysis. Our the importance of molecular approaches, in addition to whole-organism physiological processes, for a full understanding of how marine organisms respond to a changing ocean environment.



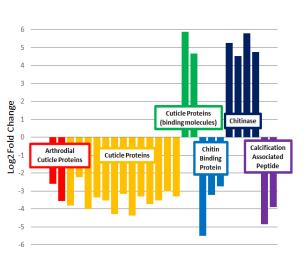


Figure 2. Response of Genes of Interest of carapace formation to elevated temperature and  $pCO_2$ . Log2 fold change of 26 GOI's within postlarvae reared under joint elevated temperature &  $pCO_2$  treatment. Gene regulation is relative to control treatments.

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# Preservation of pleopods for future molt staging in American lobster *Homarus americanus*

### From: Lydia White & Rémy Rochette

Molt status is an important biological parameter that is routinely assessed by

scientists and harvester organizations for various purposes, including to confirm the "quality" of the lobsters when selling to buyers. For example, if a lobster is close to molting it may not survive transportation and would be considered "low quality" (Lorenzon et al. 2007, Wang and Mcgaw 2014). Molt status can be assessed on the basis of external features, such as the color and hardness of the carapace, as well as attributes, such as pleopod internal morphology, hemolymph protein content, and histology. Although the assessment of external features is more convenient, assessment of internal features is less subjective and allows for identification of early molt stages that cannot be recognized on the basis of external features alone (Aiken 1973).

Pleopod staging is the most commonly used internal method to assess molt, as it is relatively easy and quick to do, minimally invasive, and enables assessment of many stages leading to moulting. Once a pleopod is sampled by removing the distal half of the endopodite or exopodite, it is typically processed within 48 hours to allow for accurate staging before the structure degrades. The ability to properly preserve pleopods would remove the need to process samples immediately after sampling, which would be particularly useful when working in remote locations and/or when engaged in extensive sampling programs. Pleopod freezing is a preservation technique that has been used with other crustaceans, including West Coast rock lobster Jasus lalandii (Isaacs et al. 2000), Hawaiian spiny lobster Panulirus marginatus (Lyle and MacDonald 1983) and the ornate rock lobster Panulirus ornatus (Turnbull 1989). There are conflicting opinions regarding the accuracy of pleopod staging after freezing (Aiken 1973, Lyle and

MacDonald 1983, Moriyasu and Mallet 1986), and the technique has not been quantitatively validated. Additionally, pleopod preservation using freezing has not been attempted for American lobster.

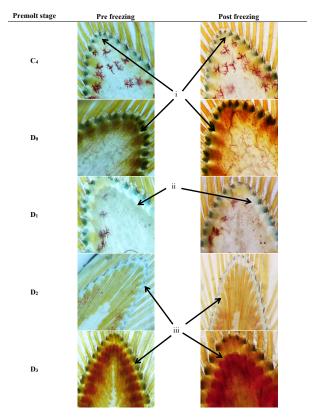


Figure 1. Photographs of American lobster pleopod clippings showing, for each of five pre-molt stages, the same pleopod pre- and post-freezing in seawater  $(C_4-D_2)$  or distilled water (D<sub>3</sub>). Key features including (i) absence or presence of cuticle separation, (ii) early seta development, and (iii) setal shaft development are still visible and retain their integrity postfreezing, potentially allowing for accurate molt staging.

We are undertaking a study to evaluate whether preserving pleopod by freezing alters the interpretation of molt status. In this study to date, a total of 456 individual exopodites and endopodites from 228 American lobsters (one pleopod per lobster) were sampled for molt staging. Lobsters ranging from 13.3 to 49.6 mm in carapace

length were collected from 11 sites in the southwest Bay of Fundy using passive biocollectors (Wahle et al. 2009). The distal end of the exopodite and endopodite on the right side of the first abdominal segment were clipped using dissecting scissors and placed in 20 ml vials filled with salt water. Pleopods were photographed using an Olympus DP72 within 24 hours of the clipping, and subsequently frozen in three different ways: (i) in empty vials (n=149), (ii) in vials filled with distilled water (n=143), or (iii) in vials filled with seawater (n=164). The pleopods were frozen for approximately eight weeks and subsequently thawed at room temperature and photographed again.

Pleopod photographs taken before and after freezing will be staged according to three scales previously described by Aiken (1973): (i) intermolt or pre-molt, (ii) 5 substages of premolt ( $C_4$  to  $D_3$ ), and (iii) 10 substages of premolt (1.0-5.5). Initial examination of the pleopod photos has shown that key structures used for molt staging are still visible post-freezing at the pre-molt stages of  $C_4$  to  $D_3$  (Figure 1). Once all photos have been staged, the technique will be validated by quantifying the error around molt staging pre- vs post-freezing, as well as overall staging error (i.e. variability around staging of the same photo multiple times).

observations Our initial from this experiment suggest that freezing pleopods for future molt staging may be a viable technique for American lobster, however further validation is required. Lobster "quality" is an important measure that affects the fishery economics directly, as well as consumers' confidence when buying (Thakur et al. 2017). The ability to preserve pleopods would allow sampling without the need for immediate processing, and hence

should increase general applicability and ease of use.

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# Utilising the settlementrecruitment relationship in harvest strategy testing of South Australian southern rock lobster (Jasus edwardsii)

### From: Adrian Linnane

Rates of puerulus settlement have been monitored in the South Australian Southern Rock Lobster (*Jasus edwardsii*) Fishery since the 1990s. The program was initiated based on the settlement-recruitment relationship observed in Western Australia where future commercial catches of *Panulirus cygnus* were predicted from settlement indices using a 3– 4 year time lag (Caputi et al. 1995). Though not as explicit, similar relationships are now evident in specific regions of some Southern Rock Lobster fisheries in both Australia and New Zealand (Gardner et al. 2001; Booth and McKenzie, 2009; Linnane et al. 2014).

Within the Northern Zone Rock Lobster Fishery (NZRLF) of South Australia, annual monitoring has been undertaken since 1996 across four sites; two sites at Port Lincoln— McLaren Point and Taylor Island—and two sites at Yorke Peninsula—Marion Bay and Stenhouse Bay (Fig. 1). The collectors are similar in design to those described by Booth and Tarring (1986) and consist of angled wooden slats that mimic natural crevice habitat (Fig. 2).

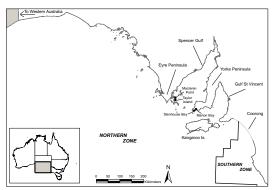


Figure 1. Northern Zone Rock Lobster Fishery of South Australia with puerulus sampling locations.

Sampling is undertaken monthly from July to October, whereby collector heads are detached from a base by a diver, covered with a mesh bag and hauled to the surface for counting of pueruli. The annual puerulus settlement index (PSI) is calculated as the mean monthly settlement on all collectors combined. This index is correlated against future recruitment indices based on previously established time lags.

Within the NZRLF, the estimated period between settlement and model estimated fishery recruitment (qR model; McGarvey et al. 1997) is four years, with both indices correlated ( $R^2 = 0.62$ ) over the period from 2000–2017 (Fig. 3). Future research now aims

to utilise this relationship to assist in the sustainable management of the resource.

In South Australia, the annual Total Allowable Commercial Catch (TACC) is set by a pre-determined decision rule as part of an overall fishery harvest strategy. Within the NZRLF, the current harvest strategy is now being reviewed. Building on the settlement-recruitment correlation, the focus is to use this relationship to test potential harvest strategy options. Specifically, the forecasting power of the PSI, in combination with the qR fishery model, will be used to gauge the impact of future recruitment on harvest strategy outcomes for different decision rules being proposed.

Projection modelling, informed by better knowledge of probable recruitment levels based on settlement-recruitment correlations, will thereby underpin the overall choice of harvest strategy selected for the fishery.



Figure 2. Collector and newly settled southern rock lobster puerulus.

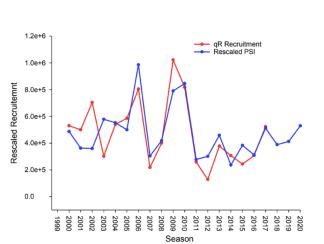


Figure 3. Correlations between NZRLF fishery model (qR) estimated recruitment and rescaled puerulus settlement index (PSI) lagged by four years.

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# The lesser-known lobster fisheries of the Andaman Islands

From: Mahima Jaini

Recently India's Andaman Islands made global news when an American missionary tried to make contact with the remote hostile Sentinelese tribe. This lesser known tribe was accessed with the help of the even more lesser-known lobster fishermen of the archipelago. The Andaman lobster fishery is a rather small enterprise, landing on an average 3.8 tonnes annually, barely making a dent in Indian lobster fisheries that contribute to 1.4% (2549 tonnes) of the global landings (Kumar et al. 2010). What the fishery lacks in quantity, it makes up in spatial extent, skilled fishing methods and the diverse communities it involves.

The Andaman Islands encompass a land area of over 6,000 km<sup>2</sup>. Population densities are low (54 people per km<sup>2</sup>) comprising of tribal groups, settler communities and migrants. The islands have tribal reserves, protected forests, sanctuaries and two marine protected areas. Tourism and natural resource extraction (timber and fish) dominate the island economy. Export based fisheries for high-value fishery commodities like groupers and lobsters started in the 1990s and now are well supported with improvements in storage and transport infrastructure (Jaini et al. 2017).

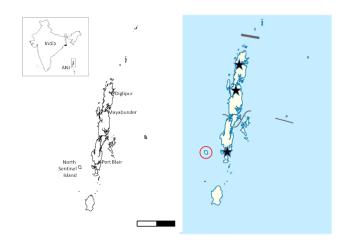


Figure 1. Map of Andaman Islands depicting the primary lobster landing centres - Diglipur, Mayabunder and Port Blair, and remote fishing locations. Inset on top left shows the location of the Andaman and Nicobar Islands (ANI) in relation to mainland India. Scale bar denotes 50 and 100 km.

The Indian government views this archipelago as a treasure trove of resources and opportunities, constantly pitching plans for luxury resort networks, trans-shipment terminals and international airports. Such development when coupled with marine resource extraction could mean havoc for the marine ecosystems that surround these picturesque isles. Sound management strategies demand a better understanding of marine resource dynamics. Unfortunately the onus of resource management continuously passes inefficiently between

various government departments and research institutions.

In 2010 a national ocean research institute published a detailed account of lobster fishing in the Andamans from 1999-2000 but lobster research since then has been meagre.



Figure 2. *P. versicolor* at a cold storage seafood processing facility in Port Blair, 2015.

Lobsters in the Andamans are mainly caught by spearfishing (3-15 m via freediving), gillnets and intertidal hand picking (Kumar et al. 2010). A recent interview I conducted last summer, as part of a preliminary scoping exercise for the design of an island ecology programme, lobster fisheries indicated that fishers have also started using compressor-based (hooka) diving increasing the targeted depth range to 30 m and dive time to over an hour. Access to good quality dive lights has further expanded their abilities allowing them to night fish for lobsters live, without external damage, thus fetching better export rates. The fishing season lasts from September to the following May or June, and fishers use large wooden boats packed with ice boxes for month-long fishing trips. Catch is sold at landing centers in North, Middle and South Andaman – namely Diglipur, Mayabunder and Port Blair on a weekly basis. The fishing fleets access far-away regions like the Invisible Banks and Sentinel Islands, and resources are even targeted inside marine parks and tribal reserves.

Of the six species caught (Panulirus verscicolor, P. penicillatus, P. ornatus, P. homarus, P. longipes, and P. polyphagus), the 2010 paper showed that catch in 1999-2000 was dominated by the pronghorn spiny lobster (Panulirus pencillatus). My personal observation in recent years seems to indicate that there might be a shift in dominance to the painted spiny lobster *P. verscicolor*. My own queries with lobster fishers suggest this shift in catch composition may be an indication of changing lobster populations or it may be driven by differences in price. *P*. pencillatus fetches an island price of 250 Rupees per kg, while *P. verscicolor* goes for 850-950 Rupees per kg.

The Andaman Islands are considered 'mini India': a melting pot of a diverse array of communities and cultures – some from the islands, others from the Indian and Burmese mainland. In the lobster fishery, different communities harvest lobsters by different means. The Karen and Bengali communities harvest via freediving and shore-based harvests, whereas the Telugu community caches them in gillnets. Fishers do not harvest individuals smaller than 300 grams as they are often rejected by the traders. Thus by combining social, ecological and economic information on the fishery linking it to other spiny lobster fisheries around the world, we hope to design effective monitoring and management strategies that ensure long-term viability of such high value commodities.

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### Mahima Jaini

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# Effects of the trematode *Cymatocarpus solearis* on Caribbean spiny lobsters

From: Tomás Franco-Bodek, Patricia Briones-Fourzán, Cecilia Barradas-Ortíz, Fernando Negrete-Soto, Rossanna Rodríguez-Canu, Enrique Lozano-Álvarez

The Caribbean spiny lobster (*Panulirus argus*) is a major fishing resource in the wider Caribbean region. These lobsters are important mesopredators in Caribbean coral reef systems, feeding on a wide variety of invertebrates and being consumed by different types of predators including loggerhead turtles (*Caretta caretta*). These predator-prey interactions are used by trophic-transmitted parasites that require sequential hosts to complete their life cycle. One such parasite is *Cymatocarpus solearis*, a digenean trematode that infects loggerhead

turtles using *P. argus* as a second intermediate host. The cercariae of *C. solearis* emerge from the body of the first intermediate host, which remains unknown (but is likely to be a gastropod), and penetrate into the body of spiny lobsters, where they encyst as metacercariae in the muscle tissue (Gómez del Prado et al. 2003) (Fig. 1). When lobsters are eaten by the definitive hosts, the trematodes mature in the digestive tract of turtles and eventually liberate the eggs with their faeces.

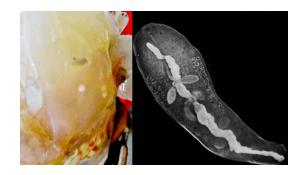


Figure 1. *Cymatocarpus solearis* cysts (white spheres) in muscle tissue of *P. argus* (right); excysted metacercaria (left)

The present study aims to determine the as vet unknown effects of C. solearis on the lobster hosts. Because many parasites have multidimensional effects on their hosts (Cézilly et al. 2013) we are comparing several traits between infected (with metacercarial cycts) and uninfected lobsters. In the field, we measured the hemolymph refraction index of lobsters (HRI, a proxy for nutritional condition once molt stage is taken into consideration) using a hand-held refractrometer. In the laboratory, we are comparing the response to conspecific chemical cues, total hemocyte count, the concentration of various components of the hemolymph chemistry and of the neurotransmitters dopamine and serotonine, the escape response, and growth rates. As this is an ongoing study, we only present preliminary results.

### Field study

In Bahía de la Ascensión (Mexico), where the prevalence of C. solearis is relatively high (Briones-Fourzán et al. 2016), we sampled 191 lobsters (40.2-110.8 mm CL) directly from commercial "casitas" deployed throughout the fishing grounds and also from the lobster catch at the landing dock. We examined lobsters for metacercarial cysts, visible in the abdominal muscles through the translucent membranes. Lobsters without visible cysts were considered uninfected. Prevalence of infection was 23.9%. HRI did not differ significantly between infected (N = 48) and uninfected individuals (N = 102) in intermolt (Student's *t* test; *t* = 0.37, gl = 148, p = 0.351).

### Laboratory experiments

Live lobsters from Bahía de la Ascensión and nearby Bahía Espíritu Santo were transported to the lab, kept in circular tanks 3 m and 2 m in diameter with a "casita" (artificial shelter) in the middle, and fed three times per week. In addition to C. solearis, lobsters in this region host the pathogenic Panulirus argus virus 1 (PaV1). Because of this, we tested a sample of hemolymph from each lobster for the presence of PaV1 using PCR assays (Huchin-Mian et al. 2013). Data from lobsters testing positive to PaV1 are discarded.

### Chemical cues

Healthy lobsters are gregarious, but tend to avoid chemical cues from both injured (Briones-Fourzán et al. 2008) and heavily PaV1-infected conspecifics (Behringer et al. 2006; Candia-Zulbarán et al. 2015). To examine potential effects of infection with *C. solearis* on lobster chemical cues, we used Ymazes to test whether focal lobsters (either infected or uninfected with *C. solearis*) avoided shelters emanating chemical cues from a stimulus lobster (either infected or uninfected) compared to shelters receiving plain seawater (control). Thus, there were four treatments: 1) uninfected focal lobster vs. uninfected stimulus, 2) uninfected focal lobster vs. infected stimulus, 3) infected focal lobster vs uninfected stimulus, and 4) infected focal lobster vs. infected stimulus. The shelter choice by focal lobsters did not significantly from random differ in treatment 1 (binomial test,  $\alpha = 0.05$ , p = 1) or treatment 2 (p = 0.6). Treatments 3 and 4 are still ongoing.

### Escape response and growth rate

To test for an effect of infection with C. solearis on the escape response of lobsters we built a channel 4.5 m in length x 40 cm in depth x 50 cm in width, where a lobster could escape (by tail-flipping, which results in a backward-swimming motion) а simulated predator attack. The data are currently being analyzed to compare several variables between infected and uninfected lobsters (delay to escape, duration of a swimming bout, distance traveled in a swimming bout, swimming velocity, and acceleration). Growth rate is also being compared between infected and uninfected lobsters that have undergone at least two molts in captivity, so as to have estimations of both size increment and intermolt period.

### Hemolymph chemistry

Using commercial kits, we measured the hemolymph serum concentration of cholesterol, total protein, albumin, and glucose. The median values of these metabolites did not differ significantly between infected and uninfected individuals (Wilcoxon T-tests, Table 1). Total hemocyte counts are yet to be completed. Table 1. Median concentrations of different hemolymph components. The medians were compared using Wilcoxon T-tests. Values between brackets are the 1<sup>st</sup> and 3<sup>rd</sup> quartiles. All values are mg ml<sup>-1</sup>.

Serum	Uninfected lobsters	Infected lobsters	p-value
component	(N = 69)	(N = 18)	
Cholesterol	4.18 [3.03, 5.36]	3.51 [2.52, 7.02]	0.912
Total Protein	3.39 [1.76, 5.83]	4.28 [1.61, 5.60]	0.859
Albumin	97.7 [72.0, 136.1]	85.0 [69.6, 132.2]	0.878
Glucose	0.194 [0.135, 0.263]	0.144 [0.119, 0.247]	0.273

### Concentration of neurotransmitters

Some parasites affect the behavior of hosts by altering the concentrations of neurotransmitters such as dopamine and serotonin (e.g. Pérez-Campos et al. 2012). Using a quantitative ELISA test, we measured the concentration of serum dopamine, which differed significantly between infected and uninfected lobsters (Wilcoxon T-test, p = 0.0012) (Fig. 2). Dopamine stimulates activity in crustaceans and is associated with escalated aggressive behavior (review in Lafferty & Shaw 2013). Therefore, higher dopamine levels may alter the behavior of infected lobsters in ways that increase their risk of predation. However, serotonin levels remain to be determined.

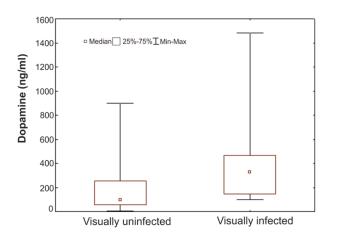


Fig. 2. Boxplot of dopamine serum concentrations in infected (right) and uninfected lobsters (left). The lower and higher boundaries of the box indicate the 1<sup>st</sup> and 3<sup>rd</sup> quartiles, respectively. The square within the box marks the median. Whiskers above and below the box indicate the range of values.

### Study caveats

Visually identifying infected lobsters entails the risk of missing infected individuals. Whereas we could only visually identify infected individuals in the field, all experimental lobsters are completely dissected once the laboratory experiments are concluded. This will reveal how many apparently uninfected individuals were actually infected, thus providing the opportunity to estimate of the sensitivity of visual identification, and will allow estimating the intensity of infection (number of trematodes per infected individual), as some of the experimental results may be related with intensity.

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## First record of the long-legged spiny lobster, *Panulirus longipes longipes*, in the Mediterranean

### From: Ehud Spanier

Levant The Basin in the eastern Mediterranean is very dynamic with respect to the invasion of non-indigenous species (NIS), especially from the Red Sea and Indo-Pacific region through the Suez Canal. This Lessepsian (or Eritreans) migration is a continuous biogeographical process responsible for the large number of tropical Indo-Pacific species that have colonized the eastern Mediterranean (Fig. 1).



Figure 1. The Lessepsian migration of Indo-Pacific marine fauna from the Indo-Pacific and Red Sea through the Suez Canal to the eastern Mediterranian.

Some have had profound effects on the indigenous biota, as well as on human activities in these waters (e.g., Spanier & Galil, 1991; Arndt et al., 2018). Galil et al. (2014) estimated that of nearly 700 multicellular NIS currently recognized from the Mediterranean Sea, fully half were introduced through the Suez Canal since its opening in 1869. Edelist et al. (2012) examined bony fish invasions in the southeastern Mediterranean and found out that a staggering 55 Indo-Pacific fish species have established permanent populations in the Mediterranean since the inauguration of the canal, more than any other marine ecosystem in the world. They also pointed out that this process was accelerating with almost half of the new arrivals (13 of 27) having established between 2000 and 2012 alone. These authors also found out that NIS fish biomass and abundance proportions in the shallow open coast have doubled in just two decades and that the present Levantine ecosystem was dominated by NIS resulting in significant declines of several indigenous species, some to near extirpation level. The invasion of fishes into the Mediterranean is mirrored by a similarly intense invasion of marine invertebrates. Galil (2011) estimated that 106 alien marine crustacean species have been recorded in the Mediterranean Sea, and many have established viable populations including many species of decapod crustacean.

Increased water temperature is assumed to facilitate invasion of non-indigenous tropical Lessepsian species from the Red Sea and the Indo-Pacific region to the Mediterranean via the Suez Canal. Recent warming of the waters and climatic changes which began in the late 1990s, permitting the invasion of species previously excluded by cooler Mediterranean waters, although additional/alternative causes such as intense fishery pressure on the indigenous biota and the recent widening of the Suez Canal should also be considered (e.g., Galil et al., 2015).



Figure 2. The exuvia of *Panulirus longipes longipes* shortly after being removed from the water (Photographed by E. Friedman).

While two species of spiny lobsters are commonly found in the Mediterranean Sea -*Palinurus elephas* (Fabricius, 1787) and *Palinurus mauritanicus* Gruvel, 1911, none of them are found in the extreme eastern and south eastern parts (Holthuis, 1991). Yet there was a single find of an Indo-Pacific lobster, *Panulirus ornatus*, in the coastal waters of Israel in 1988 (Galil et al., 1989), and here we present apparent first evidence of an additional tropical spiny lobster in the Mediterranean.

A complete fresh (with unfaded colors) single exuvia of another species of spiny lobster (Fig. 2 and 3) discovered on April 11, 2018 during a SCUBA dive by Erik Friedman who was searching with a flashlight for the common nocturnal lionfish, *Pterois miles*, a Lessepsian NIS itself. The exuvia was found at noon time approximately 1500 m off the southern coast of Haifa, the northern

Mediterranean coast of Israel, at 15 m depth hidden in the dark about 3 m under a rocky ledge. The position of the collection site (32° 49'169"N, 34° 56'571"E) is approximately 4.2 nautical miles from the opening of the nearby Port of Haifa (head of the breakwater). The geomorphology of the area is governed by cemented aeolian sandstone that forms a rocky substrate locally known as "Kurkar", is rich with crevices, ledges and caves. The bottom is composed of coarse biogenic sand and gravel. Water temperature at the site at the time of collection was 19 °C.



Figure 3. A dorsal view of the exuvia of *Panulirus longipes longipes* (Photographed by E. Spanier).

The exuvia was identified as that of a female of the long-legged spiny lobster *Panulirus longipes longipes* (A. Milne Edwards, 1868) (Spanier & Friedman, 2019). Its dimensions (CL= 97 mm, CW= 81 mm, TL = 305 mm) are typical to fully grown adult of this species (Holthuis, 1991).

Despite the increasing number of Indo-Pacific species in the Mediterranean, the vectors of NIS introduction often remain unclear. Carlton & Ruiz (2015) suggested that human-mediated transport mechanisms, primarily vessel hull fouling and ballast water, fisheries, aquaculture, oil rigs, and canals played important roles in NIS dispersal. Marine anthropogenic debris, aquarium trade and release of aquarium specimens (e.g., Mantelatto et al., 2018) and Zoochory (e.g., Guy-Haim et al., 2017) can also be considered as additional possible transport vectors. Despite the wide distribution of P. I. longipes in the Indo-West Pacific region, it has not been reported from the Red Sea and the closest area is east Africa. It is of high commercial value in Kenya where it is fished by various methods. The shipping routes from south-east Asia via the Suez Canal pass through the geographical distribution range of P. I. longipes - east Africa to Thailand, Taiwan, the Philippines and Indonesia (Holthuis, 1991). The proximity of the finding site to the northern opening of the Suez Canal and especially to the opening of the port of Haifa (as that of the finding of *P*. ornatus by Galil et al., 1988, in the same area), supports transfer of larvae, juvenile or adults in ballast water. Yet, spiny lobster larvae need oceanic clean water, and the larvae have a specific diet that could not be found in the ballast water. However, Haifa Bay supplies the proper habitats for adults of this species - clear or slightly turbid water at depths of 1 to 18 m in rocky areas (Holthuis, 1991). The similarity of other environmental conditions required by the species, such elevated water as temperatures, to those of the Indo-West Pacific region, also support the assumption that it is a Lessepsian migrant. Contrary to the *P. ornatus* recorded in this same area in the 1980s, the fresh exuvia found in the present study has no biofouling on it. This may indicate that the lobster arrived in ballast water or was released from an aquarium where biofouling activities may be minimal. The site of the finding of the exuvia, deep in a dark shelter, is typical to

molting habitats of Panulirus species (e.g., Butler et al., 2006). Thus, one assumption is that the lobster has recently molted under the ledge and left an intact exuvia. Larval stages of Panulirus spp. are known to ride large gelatinous zooplankton such as jellyfish (e.g., O'Rorke et al., 2014) and huge swarms of the Lessepsian scyphozoan jellyfish, Rhopilema nomadica, have been formed annually along the Levantine coast for more than 30 years now (e.g., Spanier & Galil, 1991; Galil et al., 2015). Thus, zoochory, the dispersal of the larvae of one species by hitch-hiking on another, is possible. Since long distance migration are known among shallow-water palinurids (e.g., Butler et al., 2006), this self-propel vector should be taken into account as well. Therefore we assume that this lobster moulted under the ledge leaving behind an intact exuvia. However, one cannot reject the possibility that the exuvia had been disposed of from an aquarium of a slow moving (so that it would have remained fully intact and undamaged) passing cruiseship or a pleasure yacht entering or exiting the close-by Haifa port. As is true of other colourful spiny lobsters, P. l. longipes is used in the aquarium trade. Thus, the issue of the source of the exuvia can be solved only when more evidence is found. If the moult was really fresh as its strong colours have indicated (although these lobsters can also keep the colour for a very long time), the adult may still be found in the vicinity. Intensive and repetitive SCUBA diving searches in the general location of the find since April 2018, as well as widespread inquiry among professional and sport fishermen in the area have been, so far, fruitless.

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# LOOKING FOR RECORDS OF NON-INDIGENOUS LOBSTERS

If you know of alien (invaded) species of lobsters recorded out of their known distribution range please email

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# **OBITUARY**

John P. Mercer 1943 - 2018



Last year saw the passing of the eminent Dr. John P. Mercer (Zoology Department, National University of Ireland, Galway). John was a pioneer of lobster research for over 40 years and contributed enormously to the biological understanding of many species, but particularly his beloved *Palinurus elephas* and *Homarus gammarus*. After graduating with a degree in Natural Sciences from Trinity College Dublin in 1966, John worked as a Scientific Officer with the Northern Ireland Fisheries division before joining the Zoology department in Galway under the leadership of Professor Padraig O'Ceidigh in 1968.

From an Occupational Health and Safety perspective, the early days of marine science on the west coast of Ireland appeared to have been precarious to say the least! As a research assistant, John was skilled in SCUBA and in later years, the story of his so-called "yellow submarine" were to become legendary. By all accounts the vehicle was a "*battery-driven, tubular, fibre-glass torpedo*" on which two divers could sit and traverse considerable distances underwater. One can only imagine the adventure (not to mention danger) associated with this underwater rodeo!

In 1973, John was awarded a Ph.D. for his thesis titled "Studies on the Spiny Lobsters (Crustacea: Decapoda: Palinuridae) of the West Coast of Ireland with particular reference to Palinurus elephas". Around the same time, the University Zoology Department established a new Shellfish Research Laboratory (SRL) at Carna, in Connemara, County Galway to which John was appointed Director. The SRL flourished under John's leadership attracting students and scientists from

around the world. Applied research projects were diverse and included breeding of European oysters, studies on the benthic ecology of the nearby Killary Harbour, cultivation of both the Japanese or Pacific abalone *Haliotis discus hannai* and the European abalone, *Haliotis tuberculata*. In addition, in what was a first for Irish waters, the SRL pioneered research into the cultivation of the purple urchin, *Paracentrotus lividus*, a native Irish species, stocks of which had been greatly reduced by overfishing.

John's ability to acquire funding to maintain the SRL, particularly during lean monetary resourcing periods, was legendary. This often involved long hours of travelling, negotiating, and late-night dining in *Morans on the Weir* where John could be found cajoling potential investors on the endless potential of urchin roe (while at same time on a call to a high ranking EU official in Brussels!).

In the early 1990s, helped by grants from the EU's Framework Programmes, the SRL secured funding to begin a major lobster (*H. gammarus*) cultivation and restocking program. Dr. Gro van der Meeren (Norwegian Institute of Marine Research) recalls a time when John was invited to a Norwegian lobster seminar on European lobster restocking at Kvitsøy:

"He made an unforgettable impression on everyone present, from his lobster enthusiasm, experience and story-telling (which almost invariably included John's mimicking of the "communicationclicks" of Palinurus elephas), to the need for a separate cottage to avoid smoke-polluting all those attending! The future of early-life lobster research in Norway and in Europe, was strongly impacted by him for years to come".

The most important outcome from the seminar was a major trans-European study, which involved collaboration between Ireland, the U.K., Norway and the Mediterranean aimed at investigating the early life history stages of the species across its geographical range. Titled *Lobster Ecology and Recruitment* (LEAR) the project involved a range of both field and experimental studies and for the first time in Irish waters, saw the "suction sampling" technique (previously pioneered by Prof. Richard Wahle from the University of Maine) applied to European benthic habitats. Attaining funding for LEAR and the arduous task of summarising the wealth of data into a final report is aptly described by Dr. Colin Bannister (formerly of Lowestoft Laboratory, U.K., and project co-investigator):

"This bold idea challenged not only us, but sceptical Brussels reviewers who did not 'get' the habitat/settlement/ behaviour concept, or the aim of lobster enhancement. I well recall long, latenight phone-ins with Carna during the 1996/7 winter, when wind and rain battered the windows at both ends, and John and I fed off each other with dogged persistence as we crafted and recrafted our text to hit the right note. We got the money in the end, started the work, and had great moments along the way, especially in Galway, where the short landing strip (!), oysters and Guinness at a meal in Eyre Square, Irish music in the Crane pub, and the roar of the Corrib in spate, are abiding memories. EPB [early benthic phase] lobsters stayed elusive, but we got the rest done (Mercer et al. 2001) and, dogged to the end, John and I spent a non-stop 48 hours at the Heathrow Holiday Inn, banging out the final reports in the fug of blue smoke from his Carroll's cigarettes".

For those involved in the project, John's charismatic leadership of the LEAR project will have long lasting memories. Who will forget his abject abhorrence (and complete disregard!) of "*No Smoking*" signs – justifying that "*rules were there to be broken*!!!"

John loved travelling and going on an overseas trip with him was always an adventure. On attending the 4<sup>th</sup> International Lobster Workshop at Sanriku, Japan, Dr. Bannister recalled the following narrative:

"On outings arranged by our generous hosts we marvelled at the scale and variety of coastal aquaculture, including the vast arrays of tanks for growing Haliotis, which greatly energised John and his ambitions for Carna. And we genuflected instinctively on seeing the first spiny lobster larva to survive in Prof. Jiro Kitaka's rearing tank. When John led us to downtown Kamaishi one night, we found that he was adept at finding refreshment in unlikely places, magically sniffing out a shop with an amazing collection of world whiskies, and beguiling the owner, who spoke no English, to allow us in for a bit. Next day John showed an enviable ability to function unaffected by the night before".

On his retirement in 2005, John settled to his beloved Inishbofin Island off the west coast of Ireland where he remained active in local conservation, in particular, the terrestrial takeover of extensive areas of the western seaboard by the Giant Rhubarb *Gunnera tinctoria*. John is much missed by his wife, Phyllis, his son Brian, his daughters Rachel and Melanie, his brother James and his beloved grandchildren. To those that knew him, John was a loyal friend, a life mentor, a kind generous soul and a true gentleman.

Ar dheis láimh Dé go raibh a anam dhílis. (May his loyal soul be at God's right hand).

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Contact Nick Caputi (southern hemisphere) or Rick Wahle (northern hemisphere) about article submissions and inquiries or corrections to the Lobster Newsletter mailing list.