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Editorial

The 37th issue of the *SPC Beche-de-mer Information Bulletin* has 23 original articles and reports on the biodiversity, fisheries, aquaculture and trade of sea cucumbers in various regions, including Papua New Guinea, Philippines, China, Brazil and Nicaragua; sea cucumber symbionts; the reproduction of some sea cucumber species; and the development of methods to better study and rear them. New *in situ* observations are also included in this issue, along with communications about upcoming conferences, and PhD dissertations that were completed this year.

The first article is from K. Barclay et al. who summarise some of the major findings of a governance analysis that was conducted to assist the National Fisheries Authority of Papua New Guinea with understanding the factors influencing the effectiveness of their revised Beche-de-mer Fishery Management Plan.

The second and third articles are from the Philippines. R. Dolorosa et al. determine the species diversity of sea cucumbers in Rasa Island Wildlife Sanctuary in addition to the catch-per-unit-effort and earnings of collectors. J.B.S. Jontila et al. provide an initial assessment on the status of sea cucumbers at Arreceffi Island in terms of species composition, abundance and distribution.

Fisheries information comes from Turkey, Brazil and Nicaragua. M. Aydın reports on the present status of the sea cucumber fishery in Turkey and J. de Souza Junior et al. on sea cucumber fisheries in northeastern Brazil. Rogers et al. draw our attention to a fishery in Nicaragua that operates from nearby Honduras, where unethical conditions occur aboard fishing vessels and where desperate poverty drives fishers to work in dangerous conditions.

S.W. Purcell and M. Wu reveal that the sandfish *Holothuria scabra* is now produced in southern China within multitrophic polyculture ponds with pearl oysters and groupers. S.W. Purcell et al. record the values of the sea cucumber "pentard" in China and determine whether market price varied as a function of the size of the products.

Works on sea cucumber reproductions come from R. Santos et al. and from V. Agudelo-Martínez and A. Rodríguez-Forero. The first team characterizes the reproductive biology of *Holothuria mammata* from Peniche, Portugal, and the second that of *Isostichopus* sp. aff *badionotus* from the Carribbean coast of Colombia.

Two original articles focus on sea cucumber symbionts. J.F. Hamel et al. reveal a new host for a species of the genus *Anoplodium*, and report on its infestation rates and negative impacts on the host, supporting its classification as parasitic. K.W. Mueller present the symbionts of the giant red sea cucumber, *Parastichopus californicus,* in Washington State (USA), with some implications for culturing the host.

F. Ducarme provides an update on the geographical range of the holothurian species *Actinopyga capillata*, first described as endemic from the Mascarene Islands, with one ectopic record from the Philippines. D. Pratas et al. show the results of four tests that aimed at determining the gender of *Holothuria forskali* without causing evisceration or consequential mortality.

This issue also reports on the processing techniques of teatfish in Fiji, on field observations on the regeneration of *Synapta maculata*, and on sea cucumbers from La Réunion, Belize and Sulawesi. The last section of this issue includes communications about workshops and conferences that were held in 2016 and some that will take place in 2017. Congratulations are expressed to Georgina Robinson from Newcastle University (United Kingdom), Guillaume Caulier from the University of Mons (Belgium), Marielle Dumestre from the University of Hong Kong (China) and Kennedy Wolfe from the University of Sydney (Australia) who recently completed their PhD dissertations of various aspects of sea cucumbers.

Igor Eeckhaut

P.S: In line with a worldwide trend to limit the impact of producing printed publications on the environment, SPC has decided to stop the production and distribution of printed copies of this and other information bulletins. The BDM bulletin is now only produced in digital format and remain accessible from SPC's website at:

http://www.spc.int/coastfish/en/publications/bulletins/beche-de-mer.html

Symbionts of the giant red sea cucumber, *Parastichopus californicus* (Stimpson, 1857), with some implications for culture of the host

Karl W. Mueller[1]

Abstract

Commercial fishing impacts on giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) populations in Washington State, USA have prompted the development of hatchery methods for and commercial-scale aquaculture of the species. As these practices grow, it will become increasingly important for culturists to be familiar with naturally occurring *P. californicus* symbionts. For the first time, all of the known symbionts of the giant red sea cucumber are briefly reviewed. Furthermore, new data are presented concerning the ecology of a commensal scaleworm and an endoparasitic snail. This information can be used by culturists and hatchery workers when establishing health management plans for and identifying possible triggers of harmful conditions affecting reared *P. californicus*.

Introduction

The giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) is fully exploited in shallow (depth ≤ 36 m relative to mean lower low water), inland marine waters of Washington State in the United States of America (USA) (Carson et al. 2016; Mueller 2016). In fact, commercial fisheries have resulted in local depletions of *P. californicus* in some management areas of the state, prompting natural resource authorities to develop aquaculture methods and stock enhancement plans for the species (PSI undated; Williams 2014). While scientists have pursued experimental aquaculture of *P. californicus* in Alaska, USA and British Columbia (BC), Canada, mostly in conjunction with other marine species (Ahlgren 1998; Paltzat et al. 2008; Hannah et al. 2013), it has not been until recently that researchers have demonstrated the plausibility of commercial-scale aquaculture of the giant red sea cucumber (DFO 2014; Royal 2016; Suhrbier et al. 2016). As the practice develops beyond experimentation, it will become increasingly important for culturists to be aware of the natural occurrence of *P. californicus* symbionts (Yanong and Erlacher-Reid 2012; Blaylock and Bullard 2014; Overstreet and Lotz 2016), especially because some symbionts have been problematic for cultured sea cucumbers elsewhere (Eeckhaut et al. 2004; Shinn et al. 2015). For the purposes of this paper, the definition of the symbiotic relationship between the giant red sea cucumber and its associated species will follow Lincoln et al. (1992); i.e. all relationships and interactions between members of two different species, including commensalism, mutualism, parasitism, and other relationships (e.g. bacterial), which has been

used similarly by others (Overstreet and Lotz 2016). Endosymbionts are referred to as *infecting* a host, whereas ectosymbionts *infest* a host (Overstreet and Lotz 2016). General terms and quantitative descriptors of symbiont populations (e.g. *prevalence* or *intensity* of), especially those of parasites, follow Bush et al. (1997). Following is the first (albeit brief) comprehensive review of the associations between *P. californicus* and its known symbionts. In addition, novel information is provided concerning the ecology of two rather charismatic symbionts of the giant red sea cucumber. In the end, some implications of these relationships are discussed for the benefit of sea cucumber culturists and hatchery personnel.

Review of giant red sea cucumber symbionts

Bacteria

Until recently, reports of bacterial infections in wild and cultured sea cucumbers have been rare in the scientific literature (Jangoux 1987a; Eeckhaut et al. 2004; Wang et al. 2004; Deng et al. 2009; Liu et al. 2010). There is no documentation of a natural occurrence of bacteriosis in *P. californicus* (DFO 2014); however, the immune response of the giant red sea cucumber has been well documented for nearly 100 years. For example, an early description of *P. californicus* coelomocyte form and function was provided by Kindred (1924). Boolootian and Giese (1958) confirmed Kindred's (1924) findings and added to the number and type of coelomocytes involved in the *P. californicus* immune response. Conversely, Hetzel (1963) reexamined the coelomic structures reported by the others, refined their descriptions of number, form and function, and hypothesized (correctly) that

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holothurian "brown bodies" were not another type of coelomocyte; rather, the yellowish-brown granules were the product of an immune reaction. Hetzel later went on to describe the formation of brown bodies in *P. californicus* and other sea cucumbers (Hetzel 1965). Dybas and Fankboner (1986) demonstrated the strong immune response of *P. californicus* to a variety of marine bacteria (gut-associated) injected into the coelomic cavity of the sea cucumber, including phagocytosis, the formation and elimination of brown bodies, the degradation of encapsulated material, and the bactericidal activity of the lysosomal enzyme, acid phosphatase. The authors surmised that the antibacterial response in *P. californicus* was a survival mechanism that coincided with the seasonal aestivation process where gut-associated bacteria might be exposed to the sea cucumber. Finally, in studying the coelomoducts overlying the rectum of *P. californicus*, Shinn et al. (1990) suggested that, besides helping maintain hydrostatic pressure within the body, the coelomoducts provided a pathway for brown bodies to exit the coelom, thereby facilitating excretion and the immune response of the giant red sea cucumber.

Nonbacterial symbionts

Like many sea cucumbers, *P. californicus* has a rich assemblage of nonbacterial symbionts — representing several taxonomic groups — associated with it (Jangoux 1987a, b). Table 1 summarizes these nonbacterial symbionts and extends Jangoux's lists (1987a, 1987b) to include protozoans and a crustacean symbiont of the giant red sea cucumber; their classifications follow those recently reported for eugregarines and coccidians (Kopečná et al. 2006; Wakeman and Leander 2012), ciliates (Lynn and Strüder-Kypke 2002; Gao et al. 2012), flatworms (Van Steenkiste et al. 2013), endoparasitic gastropods (Colgan et al. 2007), scale-bearing polychaetes (Struck et al. 2011; Norlinder et al. 2012; Norlinder 2013), and pea crabs (Ng et al. 2008; Tsang et al. 2014; Palacios-Theil et al. 2016). Some nonbacterial symbionts have been documented in the past few years or decades, whereas others have been recognized for at least a century or more (Table 1).

Eugregarines and Coccidians

Protozoan symbionts are common in holothurians (Jangoux 1987a; Eeckhaut et al. 2004); however, information on disease-forming varieties is limited (e.g. Massin et al. 1978). For example, Fankboner and Cameron (1985) described blistering on the viscera of *P. californicus* during its annual aestivation process. The blisters contained masses of sporocysts from unidentified eugregarines; their presence was considered normal for the late visceral atrophy phase. Furthermore, MacCallum et al. (2001) mentioned finding only mature oocysts of an unidentified, apicomplexan coccidian in the respiratory tree and cloaca of a captive specimen of *P. californicus* in British Columbia, Canada. Given that there was no apparent reaction to the oocysts by the host, Bower (2004a) ranked the symbiont as one of negligible regulatory significance to Canadian natural resource authorities, citing that prevention and control of the coccidian would be impractical.

Ciliates

On the other hand, with the advent of commercial-scale aquaculture elsewhere, protozoan ciliates have become potentially problematic for cultured sea cucumbers (Wang et al. 2004; Long et al. 2006). The giant red sea cucumber harbors at least two species of these symbionts (Table 1), including one scuticociliate, *Boveria subcylindrica* (Stevens, 1901), that shares the same genus as a disease-causing agent in Asia (Wang et al. 2004; Long et al. 2006). Stevens (1901) remarked that *B. subcylindrica* and a larger spirotrich ciliate, *Licnophora macfarlandi* (Stevens, 1901; but see expanded description by Balamuth 1941), inhabited the respiratory trees of all but one of the 100+ giant red sea cucumber she examined (prevalence ≥ 99%) from the vicinity of Monterey Bay, California, USA. Furthermore, she observed that the smaller *B. subcylindrica* was readily consumed by the larger *L. macfarlandi*, and that there was interannual variation in the intensities of both species. In a natural state, impacts of the ciliates on *P. californicus* are unknown (Jaeckle and Strathmann 2013). Stevens (1901) concluded that the two ciliates had no or little impact on their host, which was echoed a century later by MacCallum et al. (2001), who discovered unidentified ciliates in two captive specimens of *P. californicus* from BC, Canada. Since there were no apparent reactions to the unidentified ciliates by the host, Bower (2004b) ranked these symbionts to be of negligible regulatory significance to Canadian natural resource authorities as well, stating that prevention and control of them would be impractical, especially given the ubiquity of the unidentified ciliates.

Flatworms

Symbiotic flatworms are widespread in sea cucumbers; *P. californicus* being no exception (Jangoux 1987b). The seminal 1980s work of G. L. Shinn involving the relationships between Northeast Pacific echinoderms and their symbiotic flatworms was reviewed in detail by Jangoux (1987b), so will be only briefly discussed here for the giant red sea cucumber. There are two fully-described umagillid flatworms infecting *P. californicus*: *Anoplodium hymanae* (Shinn 1983) inhabits only the coelom, whereas *Wahlia pulchella* (Kozloff and Shinn 1987) inhabits the intestine (Table 1). The former is considered to be parasitic (Shinn 1983, 1985a, b), whereas the latter's endosymbiotic role is not clearly defined (Shinn

1986; Kozloff and Shinn 1987). Fankboner and Cameron (1985) noted a high intensity of *A. hymanae* in the coelom of the host during its annual aestivation process. A third genus of umagillid flatworm, *Ozametra*, was observed by them, but this was likely Kozloff and Shinn's (1987) *W. pulchella*. All of these authors (but especially Shinn) provide substantial detail on the taxonomy, biology and ecology of these flatworms and their relationships with *P. californicus*, including levels of infections (e.g. seasonal and ontogenetic variation), host reaction to reproductive products of the symbionts (e.g. formation and expulsion of brown bodies encapsulating flatworm eggs), and potential pathways of transmission related to life history processes of the giant red sea cucumber (e.g. feeding, seasonal aestivation, and naturally-occurring evisceration of the host).

Endoparasitic snail

The shell-less, endoparasitic snail, *Enteroxenos parastichopoli* (Tikasingh, 1961) (Fig. 1 B), was likely first observed infecting *P. californicus* more than 120 years ago (Harrington and Griffin 1897), but it would take decades before the eulimid snail was correctly identified as a new symbiont of the holothurian (Tikasingh 1960) (Table 1). Studies of *E. parastichopoli* have primarily focused on histological aspects of the gastropod (Tikasingh 1962) and its systematics (Tikasingh and Pratt 1961; Kincaid 1964). As its name implies, *E. parastichopoli* infects only the giant red sea cucumber (Tikasingh 1961).

Early reports suggest that the symbiont enters the host through the latter's alimentary tract or anus, eventually making its way out through the gut wall, and finally attaching itself to the anterior end (and coelomic side) of the host's intestine where the hermaphroditic snail develops and matures (Tikasingh 1960, 1962, 1962; Kincaid 1964). Presumably, infective stages of the parasite are evacuated from the host when *P. californicus* undergoes aestivation or evisceration (Tikasingh 1962; Kincaid 1964). Neither Tikasingh (1962) nor Kincaid (1964) detected larval *E. parastichopoli* in plankton samples; yet Kincaid (1964) anecdotally described the shelled juvenile of *E. parastichopoli*, comparing his observations with a congener of the symbiont. Canadian researchers (Cameron 1985; Cameron and Fankboner 1989) were the first to attempt an assessment of how widespread *E. parastichopoli* infections might be in juvenile and subadult giant red sea cucumbers (≤ 4 years old), and finally, in Jangoux's (1987b) review of the potential impacts of eulimid parasitism on echinoderm hosts, the author concluded that any ecological consequences for the hosts were limited. Indeed, Tikasingh (1962) suggested that the endoparasitic snail probably derives its nutrition from the coelomic fluid of the host rather than the anterior end of the intestine to which it attaches.

Scaleworm

Perhaps the most widely recognized symbiont of *P. californicus* is the scaleworm *Arctonoe pulchra* (Johnson, 1897) (Fig. 1 A). The two have been inexorably paired since the scaleworm was first identified in the 1890s (Johnson 1897; Pettibone 1953; Kozloff 1996); this is especially true for infestations in the San Juan Islands, Washington State, USA (Pernet 1999) (hereafter, San Juan Islands). *A. pulchra* is commensal also with congeners of *P. californicus* (Dimock and Davenport 1971; Lambert 1986). Early studies of *A. pulchra* focused on its behavior in the presence of the giant red sea cucumber, including the scaleworm's ability to recognize its host (Davenport 1950; Davenport and Hickok 1951; Dimock and Davenport 1971). In fact, Davenport and the others' research on *Arctonoe* spp. provided the foundation for understanding host-commensal relationships among polychaetes (Martin and Britayev 1998). Additional studies focused on the taxonomy (morphological and molecular), anatomy, reproduction and successful culture of *A. pulchra* (Pettibone 1953; Pernet 1998, 1999, 2000). And similar to *E. parastichopoli*, Canadian researchers (Cameron 1985; Cameron and Fankboner 1989)
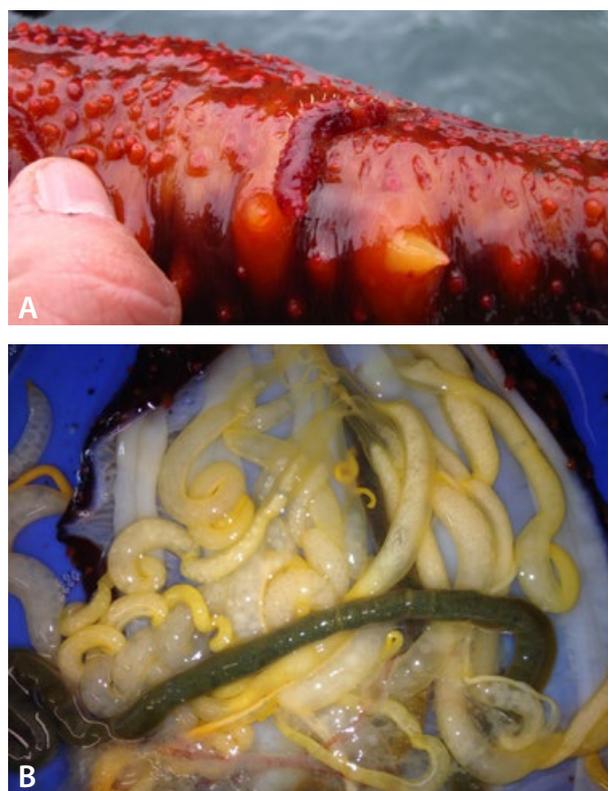
Figure 1. Two symbionts of the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857). A: The scaleworm, *Arctonoe pulchra* (Johnson, 1897), forms a commensal relationship with *P. californicus*. B: The shell-less snail, *Enteroxenos parastichopoli* (Tikasingh, 1961), forms an endoparasitic relationship with its host. Here, numerous yellow, egg-laden *E. parastichopoli* are attached to the anterior portion of the intestine of *P. californicus*.

Table 1. Nonbacterial symbionts of the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) classified to lowest taxonomic rank possible based on their original description in the scientific literature. Taxonomic ranks above genus and species were based on recent molecular phylogenies for those groups of organisms listed (see text for references).

Kingdom	Phylum	Class	Order	Family	Genus and species	Type	Host site	Reference(s)
Protozoa	Apicomplexa	Conoidasida	Eugregarinorida	NA	NA	Unidentifed eugregarine	Viscera	Fankboner and Cameron (1985)
Protozoa	Apicomplexa	Conoidasida	Eucoccidiorida	NA	NA	Unidentified coccidian	Respiratory tree; cloaca	MacCallum et al. (2001)
Protozoa	Ciliophora	Spirotrichea	Licnophorida	Licnophoridae	*Licnophora macfarlandi*	Spirotrich ciliate	Respiratory tree	Stevens (1901); Balamuth (1941)
Protozoa	Ciliophora	Oligo-hymenophorea	Thigmotrichida	Boveriidae	*Boveria subcylindrica*	Scuticociliate	Respiratory tree	Stevens (1901)
Protozoa	Ciliophora	NA	NA	NA	NA	Unidentified ciliates	Respiratory tree	MacCallum et al. (2001)
Animalia	Platyhelminthes	Rhabditophora	Rhabdocoela	Umagillidae	*Anoplodium hymanae*	Flatworm	Coelom	Shinn (1983)
Animalia	Platyhelminthes	Rhabditophora	Rhabdocoela	Umagillidae	*Wahlia pulchella*	Flatworm	Intestine	Kozloff and Shinn (1987)
Animalia	Annelida	Errantia	Phyllodocida	Polynoidae	*Arctonoe pulchra*	Scaleworm	Exterior body wall (i.e. skin)	Johnson (1897)
Animalia	Mollusca	Gastropoda	Sorbeoconcha	Eulimidae	*Enteroxenos parastichopoli*	Endoparasitic snail	Intestine	Harrington and Griffin (1897); Tikasingh (1961)
Animalia	Arthropoda	Malacostraca	Decapoda	Pinnotheridae	*Opisthopus transversus*	Pea crab	Cloaca	Rathbun (1894); Rathbun (1918)

NA = not available.

were the first to attempt an assessment of how widespread *A. pulchra* infestations might be in juvenile *P. californicus*. Lastly, fecal pellet analyses revealed that, while infesting *P. californicus*, *A. pulchra* fed mostly on crustaceans and polychaetes (Pernet 1998). Approximately one-third of scaleworms examined by Pernet (1998), however, contained unidentified tissue; he found no indication of the symbiont feeding on its host (e.g. giant red sea cucumber ossicles were not observed in fecal pellets).

Pea crab

At the close of the 19th century, a small crustacean was discovered infesting a large gastropod in southern California, USA, which is the southern extent of the range of *P. californicus* (Lambert 1986). The pinnotherid crab *Opisthopus transversus*, was formally described and named by Rathbun (1894), who went on to provide additional details on the sexual dimorphism of the pea crab and a host list comprising three additional species, including *P. californicus* (Rathbun 1918). Decades later, the relationship between *Parastichopus* and *O. transversus* was confirmed by others (Ricketts and Calvin 1968; Schmitt et al. 1973), the pea crab's range was expanded (Hopkins and Scanland 1964), and the list of host species nearly quadrupled in number (Beondé 1968). From the literature, it can be inferred that: 1) *O. transversus* is the only pinnotherid crab infesting *P. californicus* (Schmitt et al. 1973); 2) once a symbiotic relationship is established, *O. transversus* displays a strong affinity for its host (Ricketts and Calvin 1968); 3) host selection may be linked to host size (e.g. small or young pea crabs infest small hosts, and so on; Hopkins and Scanland 1964); 4) more than one host may be required to complete the life history of *O. transversus*; and 5) different hosts may satisfy different nutrient requirements for the pea crab to develop and reproduce successfully (Beondé 1968; Campos 2016). Alternatively, *O. transversus* may just be experiencing a "Goldilocks moment" in time where, evolutionarily, the species is new to symbiosis and is still determining which host is "just right" (sensu Beondé 1968).

Symbionts and sea cucumber culture: Gleaning new insights by extending the work of others

Surveys of the prevalence of *P. californicus* symbionts in the wild are few. Besides Shinn's (1985) work with the flatworm *A. hymanae*, Cameron (1985) (and later, Cameron and Fankboner 1989) provided useful information on the prevalence of *A. pulchra* and *E. parastichopoli* associated with juvenile and subadult giant red sea cucumber (≤ 4 years old) from BC, Canada; however, the latter's results were based on small sample sizes from a limited number of localities. Recently, Mueller (2016) had the opportunity to collect and inspect a greater number of *P. californicus* of varying sizes and ages,

including mature individuals (≥ 5 years old), compared with Canadian researchers. Because his study plan included dissecting every sea cucumber collected for sex determination and maturity, Mueller (2016) was able to check for the prevalence of symbionts across a wide variety of host ages and sizes. The remainder of this paper presents data collected ancillary to a study of the fishery biology of the giant red sea cucumber from the San Juan Islands (Mueller 2016). It is intended to extend the symbiont works of others (e.g. Cameron 1985; Cameron and Fankboner 1989) and to fill in some gaps in our understanding of the ecology of *A. pulchra* and *E. parastichopoli*. A discussion follows on how this information may be of use to those participating in the nascent aquaculture of *P. californicus*.

Materials and methods

Mueller's study (2016) took place in the San Juan Islands where *P. californicus* is abundant relative to other marine areas of Washington State (Bradbury et al. 1998; Carson et al. 2016). The holothurian was collected at several points within four regions (west to east): Upright Channel, Rosario Strait, Bellingham Channel, and the junction of Bellingham, Samish, and Padilla bays (hereafter, "Bays" or Vendovi Island) (Table 2).

Using the same voluntary minimum size limit adopted by many commercial harvest divers as a guideline to retain *P. californicus* (i.e. no shorter than ~5 cm on either side of a gloved hand or ~20 cm whole, contracted length), 50 giant red sea cucumber were collected by divers twice monthly from 7 May 2014 to 15 October 2014, and then again from 17 March 2015 to 28 May 2015. Individual *P. californicus* were placed inside ziplock plastic bags and stored on ice inside a large cooler until being processed the following day at a wet laboratory located on the Northwest Indian College (NWIC) campus in Bellingham. At the NWIC wet laboratory, counts of *A. pulchra* and *E. parastichopoli* were recorded opportunistically for each sea cucumber while other biological data were being gathered (Mueller 2016). The symbionts are relatively large compared with others of the giant red sea cucumber, and are easily identified by an observer without the aid of magnification (Fig. 1 A and B); hence, these qualities simplify determining their presence or absence in the field or laboratory. There was no need to distinguish *A. pulchra* from other species of *Arctonoe* because it is the only scaleworm associated with *P. californicus* in this region of the host's range (Pettibone 1953; Pernet 1999). Furthermore, no indication of size or life stage was recorded for the scaleworm and endoparasitic snail.

By the end of the study, divers had collected 850 *P. californicus* throughout the central-east San Juan

Table 2.   Localities where the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) was collected by divers in the San Juan Islands, Washington State, USA during 2014 and 2015. Depth range (m) of the collection refers to that as per diver depth gauge (uncorrected for tidal elevation), whereas average depth (m) of the collection refers to that relative to mean lower low water (MLLW).

Region	Collection point	Latitude	Longitude	Depth range (m, uncorrected)	Average depth (m, MLLW)	No. sea cucumber
Upright Channel	Canoe Island	48°33.561'N	122°55.438'W	6.1 – 15.2	10.7	50
Upright Channel	Canoe Island	48°33.675'N	122°55.433'W	5.5 – 12.2	7.0	50
Upright Channel	Lopez Island	48°33.899'N	122°53.529'W	6.1 – 19.2	11.3	50
Upright Channel	Lopez Island	48°33.913'N	122°53.526'W	6.1 – 24.4	12.8	50
Rosario Strait	Orcas Island	48°36.087'N	122°48.032'W	4.6 – 21.3	11.6	100
Rosario Strait	Blakely Island	48°35.028'N	122°47.930'W	12.2 – 20.4	14.0	50
Rosario Strait	Cypress Island	48°35.867'N	122°43.885'W	33.5 – 36.6	33.5	50
Rosario Strait	Cypress Island	48°36.184'N	122°43.542'W	4.9 – 12.2	9.8	50
Bellingham Channel	Cone Islands	48°35.564'N	122°41.021'W	6.1 – 12.2	9.1	77
Bellingham Channel	Cone Islands	48°35.520'N	122°40.579'W	3.4 – 15.2	8.5	100
Bellingham Channel	Sinclair Island	48°36.642'N	122°40.768'W	5.5 – 13.7	5.2	50
Bellingham Channel	Sinclair Island	48°36.458'N	122°39.983'W	6.1 – 12.2	9.1	23
"Bays"	Vendovi Island	48°36.821'N	122°36.886'W	6.1 – 21.3	10.4	150

Islands at average depths ranging from 5.2 m to 33.5 m (mean lower low water) (Table 2). Frequency distributions of *A. pulchra* and *E. parastichopoli* were calculated for host collection date and locality, host sex, and age and size of the giant red sea cucumber. Symbiont prevalence was determined after Bush et al. (1997) as the number of hosts infected or infested with one or more individuals of a symbiont species divided by the number of hosts examined for that species. Proportional prevalence of the symbionts were then plotted at three levels (0, 1, and ≥ 2 organisms per sea cucumber) for each of the factors except host sex. Symbiont intensity was also determined after Bush et al. (1997) as the number of individuals of a symbiont species in a single infected or infested host. Age and size of *P. californicus* were determined using methods described by Mueller (2016).

Results

The commensal scaleworm *A. pulchra* infested up to 70% of *P. californicus* collected in 2014 and 2015. The intensity of an infestation was no more than three scaleworms per sea cucumber. Proportional prevalence of *A. pulchra* was ostensibly influenced by *P. californicus* collection date and locality, and age and size of the host (Figs. 2–4). For example, proportional prevalence of *A. pulchra* increased during the spring of both study years, peaking in midsummer 2014 and again in mid-fall 2014 (Fig. 2A). Between host collection localities, the proportional prevalence of *A. pulchra* varied by as much as 0.2. While no clear longitudinal trend was observed in sea cucumbers with one scaleworm attached, there

was a slight increase in the proportional prevalence of *A. pulchra* at higher densities (i.e. ≥ 2 polychaetes per sea cucumber) moving from west to east in the San Juan Islands (Fig. 3A). In addition, the proportional prevalence of *A. pulchra* increased with the age and size of *P. californicus* collected (Fig. 4A); however, the sex of *P. californicus* did not appear to greatly influence the proportional prevalence of the scaleworm. Indeed, 179 of 364 female *P. californicus* were infested by at least one *A. pulchra* (proportional prevalence = 0.49), whereas 150 of 369 male *P. californicus* were infested by at least one scaleworm (proportional prevalence = 0.41). Lastly, at least one *A. pulchra* infested 60 of 117 giant red sea cucumbers of unknown sex (proportional prevalence = 0.51).

The shell-less, endoparasitic snail *E. parastichopoli*, on the other hand, infected up to 30% of *P. californicus* collected in 2014 and 2015. The intensity of *E. parastichopoli* infections were variable; usually, there were no more than one or two endoparasitic snails per sea cucumber, but intensities as high as 42 per sea cucumber were observed (Fig. 1B). Both juvenile and adult stages of the symbiont comprised the highest intensities of infection, whereas lower intensities were characterized by the presence of adult stages only. Like *A. pulchra*, the proportional prevalence of *E. parastichopoli* appeared to be influenced by *P. californicus* collection date and locality, and age and size of the host (Figs. 2–4). For example, the proportional presence of *E. parastichopoli* increased slightly during spring, but decreased mid-summer 2014, and decreased further by the last sampling date in fall 2014 (Fig. 2B). In terms of host collection locality (Table 2), the highest proportional
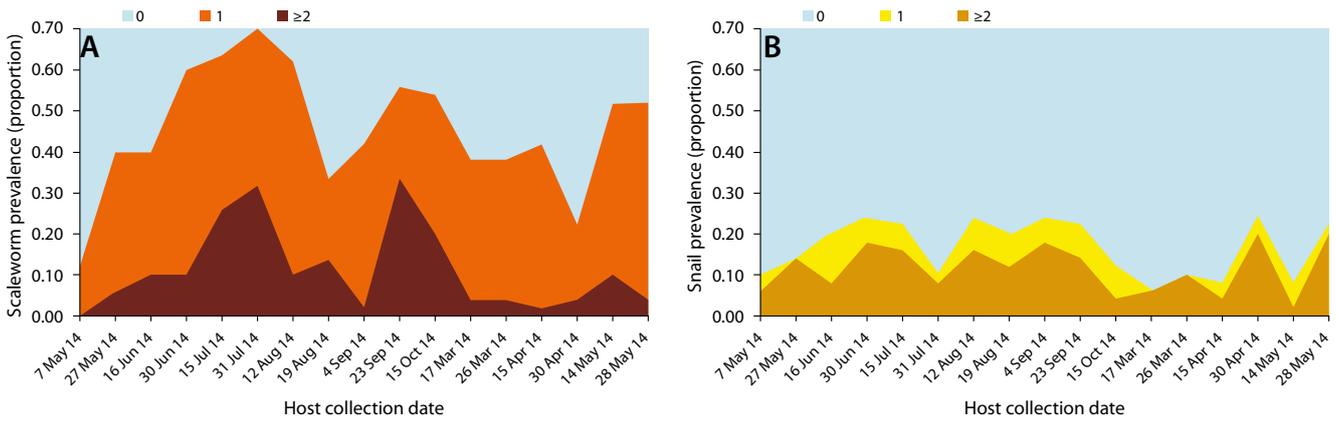
Figure 2. Proportional prevalence of symbionts of the giant red sea cucumber *Parastichopus californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host collection date. Fifty giant red sea cucumbers were collected on each date. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.
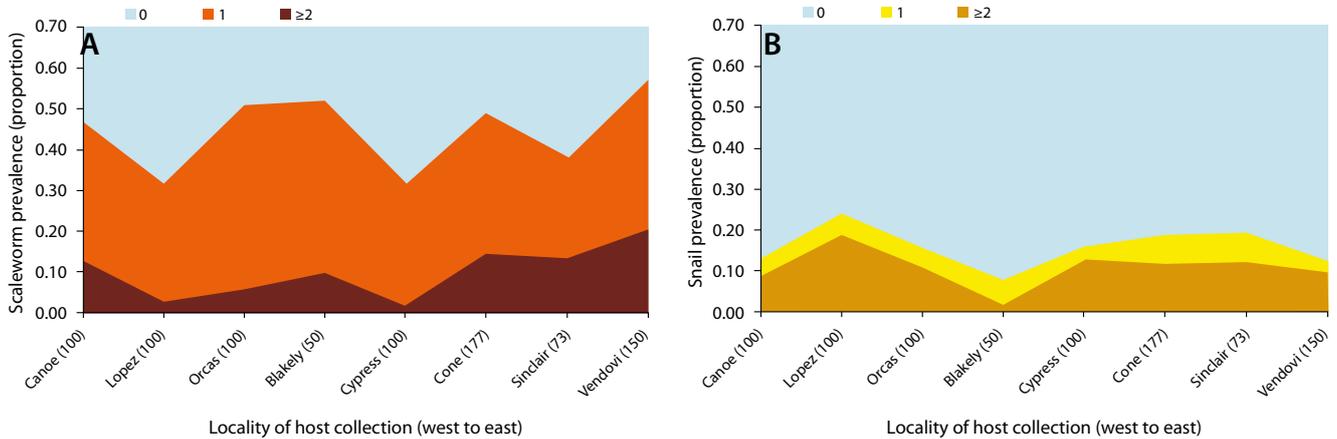
Figure 3. Proportional prevalence of symbionts of the giant red sea cucumber *P. californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host collection locality (ordered west to east). The number of giant red sea cucumbers collected by locality is indicated parenthetically. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.
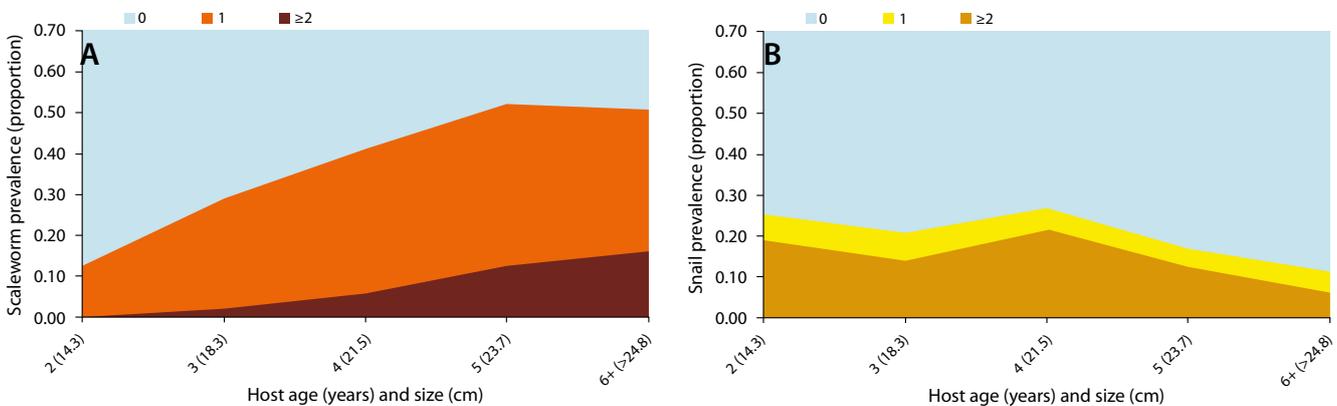
Figure 4. Proportional prevalence of symbionts of the giant red sea cucumber *P. californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host age (years) and size (cm, indicated parenthetically). The numbers of giant red sea cucumber collected for each age and size group were as follows: age 2, *n* = 16; age 3, *n* = 107; age 4, *n* = 158; age 5, *n* = 161; and age 6+, *n* = 408. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.

prevalence of endoparasitic snails occurred at Lopez Island (Upright Channel) and in the vicinity of Sinclair and the Cone islands (Bellingham Channel; Fig. 3B). And while the proportional prevalence of *E. parastichopoli* in immature *P. californicus* (age = 2–4 years) fluctuated between 0.20 and 0.25, the proportional prevalence of endoparasitic snails in mature sea cucumbers (age ≥ 5 years) fell below 0.15 (Fig. 4B). Regarding sex of the host, 50 out of 364 female *P. californicus* were infected with at least one endoparasitic snail (proportional prevalence = 0.14), whereas 56 of 369 male *P. californicus* were infected with at least one *E. parastichopoli* (proportional prevalence = 0.15). In terms of giant red sea cucumbers of unknown sex, 34 out of 117 were infected with at least one endoparasitic snail (proportional prevalence = 0.29).

Ultimately, irrespective of host collection date and locality, and age and size of *P. californicus*, there appeared to be an inverse relationship between the proportional prevalence of the commensal scaleworm and the proportional prevalence of the endoparasitic snail: whenever or wherever there was an increase in the proportional prevalence of *A. pulchra*, there was a subsequent decrease in t heproportional prevalence of *E. parastichopoli* and vice versa (Figs. 2–4).

Discussion

At present, the symbiont community of the giant red sea cucumber (Table 1) does not appear to affect its host too profoundly in the wild (Stevens 1901; Tikasingh 1962; Fankboner and Cameron 1985; Pernet 1998; Bower 2004a, b); however, when placed into an aquaculture setting, one or more of the naturally occurring symbionts of *P. californicus* could become pathogenic or become a pathway or vector for disease (Blaylock and Bullard 2014; Shinn et al. 2015; Overstreet and Lotz 2016). For example, Wang et al. (2004) reported high intensities of a protozoan ciliate inhabiting the respiratory tree of cultured *Apostichopus japonicus* (Selenka, 1867) [likely *Boveria labialis* (Ikeda and Ozaki, 1918)] that weakened the host and occasionally led to the ejection of the sea cucumber's coelomic contents. In fact, Long et al. (2006) reported secondary bacteriosis in *A. japonicus* as a result of *B. labialis* infections. Furthermore, crustaceans (Brachyura, Copepoda, and Isopoda) living in sympatry with cultured *A. japonicus* and *Holothuria scabra* (Jaeger, 1833) were identified as potential sources of high mortality in the sea cucumbers (Wang et al. 2004; Lavitra et al. 2009). Moving forward, culturists of *P. californicus* should be particularly aware of the scuticociliate *B. subcylindrica*, and in the southern extent of the host's range, the pea crab *O. transversus*. Although the latter was described as leaving

its host periodically to forage (Beondé 1968), the dynamics between host and symbiont could change in an intensive culture setting.

Prior to this study, data on the natural prevalence of *A. pulchra* were limited. For example, Cameron (1985) (and later, Cameron and Fankboner 1989) collected and inspected between 11 and 42 juvenile *P. californicus* (age ≤ 1 year; whole, contracted length < 2 cm) from each of 14 samples taken across four localities in BC, Canada. The author(s) did not report the total number of juvenile giant red sea cucumbers collected, nor did they report the number collected by locality. It can be assumed, however, that the *minimum* total number of juvenile giant red sea cucumbers collected by them was ≥ 154 (i.e. 11 sea cucumbers X 14 samples). Of all these, only 3 of the 42 juvenile giant red sea cucumbers collected during one trip to one locality were infested with a single, small *A. pulchra* (proportional prevalence = 0.071). Extending this exercise to the regional scale (i.e. all four localities combined), the proportional prevalence of *A. pulchra* on juvenile *P. californicus* can be estimated as the number of infested giant red sea cucumbers ($n = 3$) divided by the total number of holothurians from all 14 samples (n range = 11–42 *P. californicus* per sample); or, put another way, the estimate of regional proportional prevalence of the scaleworm on juvenile giant red sea cucumbers during Cameron's (1985) study was between 0.005 [= 3/(42 X 14)] and 0.019 [= 3/(11 X 14)]. In contrast, Pernet (1998) noted a 10-fold increase (or more) in the proportional prevalence of *A. pulchra* (= 0.20) infesting adult-size *P. californicus* from the San Juan Islands, yet both of these examples fall short of the summertime peak (proportional prevalence = 0.70) reported in the present study. Such spatiotemporal differences in the prevalence of symbionts have been widely reported throughout the animal kingdom (e.g. Martin and Britayev 1998; Timi and Poulin 2003; Hoffmann et al. 2016).

Data on the natural prevalence of *E. parastichopoli*, while also limited, have at least been better documented than those for the scaleworm. For example, Harrington and Griffin (1897) reported that 3 of the 30 or 40 *P. californicus* that were examined were infected by endoparasitic snails (proportional prevalence = 0.07–0.10), while Tikasingh (1960) found that just 14 out of 276 giant red sea cucumbers examined in mid-summer to early fall were infected by *E. parastichopoli* (proportional prevalence = 0.05). Furthermore, Jangoux (1987b), citing Lützen's (1979) findings, tabulated intensities of approximately three *E. parastichopoli* per host in 37 out of 244 *P. californicus* examined (proportional prevalence = 0.15), whereas Cameron and Fankboner (1989) reported a proportional prevalence of 0.09 during August 1983 (6 out of 63 subadult *P. californicus* aged 2–4 years) inspected were infected by *E. parastichopoli*, and no

endoparasitic snails were found in 36 giant red sea cucumbers examined in October 1983 at the onset of aestivation following the visceral atrophy phase. All of these findings are consistent with those from various collection dates and localities and host ages and sizes reported in the present study.

The inverse relationship between *A. pulchra* and *E. parastichopoli* is particularly intriguing. This is the first study to provide evidence of a possible interaction between the two symbionts. Early research on the endoparasitic snail suggested that the symbiont entered *P. californicus* either orally or anally, but mostly while the host fed on the earliest life stages of *E. parastichopoli* (Tikasingh 1960, 1961, 1962; Kincaid 1964). Hypotheses regarding other modes of host–symbiont contact between the giant red sea cucumber and the endoparasitic snail were never made. Only in the past decade was an alternative documented: Altnöder et al. (2007) provided photographic evidence of microscopic, shelled larvae of an endoparasitic snail attached to the skin of its holothurian host. Regarding the possible interaction between *A. pulchra* and *E. parastichopoli*, it is proposed that the scaleworm preys on the shelled larvae of the endoparasitic snail when the former encounters the latter attached to the skin of *P. californicus*. The two symbionts might also encounter each other in the buccal area of the host where *A. pulchra* is often found (Pettibone 1953). Indeed, the scaleworm readily feeds on larval bivalves and gastropods (Mollusca) in a laboratory setting (Pernet 2000); hence, predator-prey dynamics might contribute to the observed inverse relationship between the two symbionts of *P. californicus*.

Interspecific interactions among symbionts have been investigated in both laboratory and natural settings (Pugachev 2000; Timi and Poulin 2003; Fenton et al. 2014; Hoffmann et al. 2016). One example of an obvious interaction between symbionts of *P. californicus* is Stevens' (1901) record of the ciliate *L. macfarlandi* preying directly upon the smaller ciliate *B. subcylindrica* within the respiratory tree of the host. Conversely, Timi and Poulin (2003) found that changes in a host's symbiont community structure were due mostly to abiotic factors rather than interactions between symbionts. Fenton et al. (2014) cautioned that several underlying factors at the logical, temporal, and spatial scale might contribute to observed variation in symbiont prevalence and intensity that would otherwise be attributed to interspecific interactions. These authors suggested experimental and statistical approaches that would help separate interspecific interactions from other factors, potentially improving their detection. Additional causes for the observed relationship between *A. pulchra* and *E. parastichopoli* may, therefore, be revealed by applying more rigorous analytical methods *sensu* Fenton et al. (2014).

Until such work is completed, though, it is recommended that the commensal relationship between the giant red sea cucumber and *A. pulchra* be maintained in future aquaculture settings. In this way, predation by *A. pulchra* might serve as a natural prophylactic treatment for parasitosis, thereby reducing the risk of high intensity infections of *E. parastichopoli*. Laboratory cultures of the scaleworm (Pernet 1998, 1999, 2000) could even be maintained for this purpose. Given the evidence presented here for the probable interaction between the two symbionts at multiple scales (time, space and host), this simple measure should be a prudent element of any health management plan for *P. californicus* culture. Natural treatment of a pest via predation in sea cucumber aquaculture is not without precedent. For example, Lavitra et al. (2009) described intentional introductions of a carnivorous fish into commercial sea cucumber ponds to successfully eliminate a deleterious, pathogenic crustacean (Isopoda). And should the ciliate *B. subcylindrica* ever become as problematic to giant red sea cucumber culture as its congener, *B. labialis* is to *Actinopyga japonicus* culture, might predation by the larger ciliate *L. macfarlandi* be a possible solution?

To summarize, this paper provides the essential first step of reviewing known symbionts of the giant red sea cucumber and delves into the ecology of two of them on multiple levels (time, space and host), providing some possible directions for future research. For example, does the higher proportional prevalence of *E. parastichopoli* infecting *P. californicus* of unknown sex (0.29) vs those of male and female giant red sea cucumbers (0.15 and 0.14, respectively) indicate a negative impact on reproductive capacity of the host? Current knowledge of the relationship between endoparasitic gastropods and their echinoderm hosts suggests this may be unlikely (Jangoux 1987b); still, it is difficult to imagine that a moderate to severe infection of *E. parastichopoli* (Fig. 1B) has no ecological consequences for *P. californicus*. And what drives higher the proportional prevalence of *A. pulchra* in mature *P. californicus* (≥ 5 years old) compared with younger giant red sea cucumbers? The host's body size? The host's reproductive status?

In conclusion, the diversity of symbionts associated with the giant red sea cucumber will challenge future *P. californicus* culturists and hatchery workers with predicting the effects of disease or pest outbreaks and subsequent treatment protocols (Fenton et al. 2014; Shinn et al. 2015). While knowing the symbiont composition in *P. californicus* (Table 1) is an essential step in "biosecurity" (i.e. minimizing the risk of introducing or transmitting disease or pest agents) of the host species (Yanong and Erlacher-Reid 2012), other lessons to be learned include improved understanding of how symbiont

diversity affects infections or infestations, how sea cucumber culture affects genetic diversity of *P. californicus* (and subsequently, its relationships with symbionts), natural patterns in symbiont communities across different scales, and symbiont interactions at the host level (Johnson et al. 2015). This information can be used by culturists and hatchery workers when establishing health management plans for, and identifying possible triggers of, harmful conditions affecting reared *P. californicus* (Wang et al. 2004; Blaylock and Bullard 2014; Overstreet and Lotz 2016).

Acknowledgements

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