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DETECTION AND PRELIMINARY EVALUATION OF NATURAL ENEMIES FOR POSSIBLE BIOLOGICAL CONTROL OF THE NORTHERN PACIFIC SEASTAR, *ASTERIAS AMURENSIS*

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SUMMARY

Samples of the northern Pacific seastar, *Asterias amurensis*, from populations in central and northern Japan were examined to detect parasites that might have the potential to act as biological control agents for the seastar in Australian waters. The survey concentrated on detecting dendrogastrid ascothoracidan crustaceans, eulimid gastropods, the ciliate *Orchitophrya stellarum*, and the copepod *Scottomyzon gibberum*. 489 seastars from 19 sites in northern Honshu and Hokkaido were examined for parasites. No dendrogastrids or eulimids were detected. A single male seastar from Nemuro (Hokkaido) was infected with the ciliate *O. stellarum*; this represented 6.2% of all male starfish examined from this locality. Compared to published descriptions this represents a low to moderate infection rate. The copepod *S. gibberum* was detected in most samples from Mutsu Bay north although sampling at southern sites may not have been adequate to detect the parasite. The scaleworm *Arctonoe vittata* was not found on seastars from central Japan and was uncommon at most northern sites. A preliminary evaluation of these parasites suggests that *O. stellarum* merits further investigation as a potential control agent because of its impact on male fertility. The potential for *S. gibberum* to act as a biological control agent appears less than that of the ciliate and further studies are needed to assess the effects of the copepod on seastar survival. Both these parasites are relatively cryptic and it is recommended that Tasmanian populations of *A. amurensis* be examined to establish whether or not these or related species are already present in Australian waters. It is also recommended that a study be made of high density "outbreak" populations of *A. amurensis* in Japanese waters to assess whether the characteristic rapid decline of these populations is associated with the presence of infectious diseases.

INTRODUCTION

Since its introduction into Tasmanian waters in the late 1970s or early 1980s, the northern Pacific seastar, *Asterias amurensis*, has become extremely abundant in the Derwent Estuary and has spread into the adjacent D'Entrecasteaux Channel and Storm Bay areas (Davenport & McLoughlin 1993, Buttermore *et al.* 1994, Morrice 1995). The establishment of this large, generalist predator (Hawkes & Day 1993) in southern Tasmanian waters has raised serious concerns in relation to its potential to damage lucrative fisheries, impede the development of mariculture, and significantly alter the structure of natural benthic communities (Turner 1992). In response to these concerns, the Centre for Research on Introduced Marine Pests (CRIMP) has established *A. amurensis* as a priority candidate for possible biological control.

Following the general guidelines proposed for the biological control of marine species (Lafferty & Kuris 1994, 1995), Japanese populations of the seastar were examined to detect and initiate the evaluation of candidate biological control species. The survey concentrated on parasites; predators were not evaluated because of there likely impacts on native Tasmanian animals. Prior to this investigation, there was scant pertinent information on parasites of this seastar. Literature review, however, suggested four possible candidates: dendrogastrid ascothoracidan crustaceans, eulimid gastropods, the ciliate *Orchitophrya stellarum*, and the copepod *Scottomyzon gibberum*.

Dendrogastrids are of considerable interest because they are known parasitic castrators of seastars (Tyler & Pain 1982, Tyler *et al.* 1984). Eulimids have also been reported to affect seastar reproduction (Warén & Lewis 1994). The ciliate *Orchitophrya stellarum* has been reported to castrate males of other species of *Asterias* (Jangoux 1987, Bouland & Jangoux 1988); its effects on females are little studied. Some studies indicate high prevalences of these ciliates associated with low abundance of seastars, including *A. rubens* (Vevers 1951, Claereboudt & Bouland 1994). The copepod *Scottomyzon gibberum* causes lesions at the bases of spines and pedicellaria that can become necrotic (Rottger 1969).

The scanty published record of parasitological investigations of *A. amurensis* is unlikely to be due to a lack of opportunity to observe parasites of this seastar as this species is commonly used for research on developmental biology in Japan. Large parasites such as dendrogastrids or eulimids, if present, would almost certainly have been observed and reported. Indeed, eulimids (*Parvioris astropectenicola*) have been reported from Tokyo Bay (Warén 1981). The absence of reports of ciliates and copepods could be due to oversight (they are small and often inconspicuous), negative records are often not published, or these species do not parasitise *A. amurensis* in Japan. Kim (1992) reported a copepod as *S. gibberum* from *A. amurensis* in Korea.

Two other types of parasites might also be encountered in a parasitological investigation of *A. amurensis.* Myzostomida similar to *Asteriomyzon asteriae* have been recovered as ectoparasites of *Asterias richardi* in the Mediterranean and *Asteromyzostomum* spp. are found in pyloric caeca of some other seastars (Jangoux 1990). Also, pterastericolid turbellarians are parasitic in pyloric caeca of various seastars (Cannon 1975). These parasites were included in the study although no pathological sequelae have been reported and their potential as control agents is likely to be nil.

METHODS

To afford the highest likelihood of detecting unknown and possibly rare parasites, we opted for a search strategy to sample relatively few seastars (~20) from as many sites and different localities as feasible. Accordingly, we visited 8 universities, marine laboratories and field stations from 6–28 July 1995 and examined seastar from 19 sites. Collection methods were variable and included by hand collecting in the intertidal zone, free-diving, SCUBA diving, grappling with rakes, fish-baited traps, scallop dredges (commercial fisheries), and scallop spat collectors. At one locality (Tateyama), seastars had been held in the laboratory for three months; at other sites, seastars were freshly caught (held less than 5 days).

At each site, we used all available seastars or, if too many were available for a complete examination, we haphazardly selected a subset for analysis. We recorded size (arm length to the centre of the disk) for all arms, colour, sex (from examination of gonads) and any evident abnormalities. Seastars were dissected by cutting around the periphery to separate the oral from the aboral surfaces. The external surfaces and the internal organs were thoroughly examined for macroparasites and lesions. Coelomic fluid was drained and a drop of fluid was microscopically examined (100x) for microbial infections. Squash preparations of a sample of digestive gland and gonadal tissue were microscopically examined as were all lesions or other abnormalities. Under this general protocol 40–60 seastars could be examined in a full working day.

Following discovery of the copepod, *S. gibberum*, we initiated additional procedures: an additional 4 seastars were anaesthetised in 10% ethanol in sea water and the washings were decanted and carefully examined for copepods which were counted, removed and preserved (along with other organisms in the washings). At two sites, at least 10 additional seastars were freshly collected (by hand in the intertidal zone and by diving in shallow subtidal habitats), individually bagged and subjected to an alcohol bath. Copepods and other animals in the washings were counted and preserved.

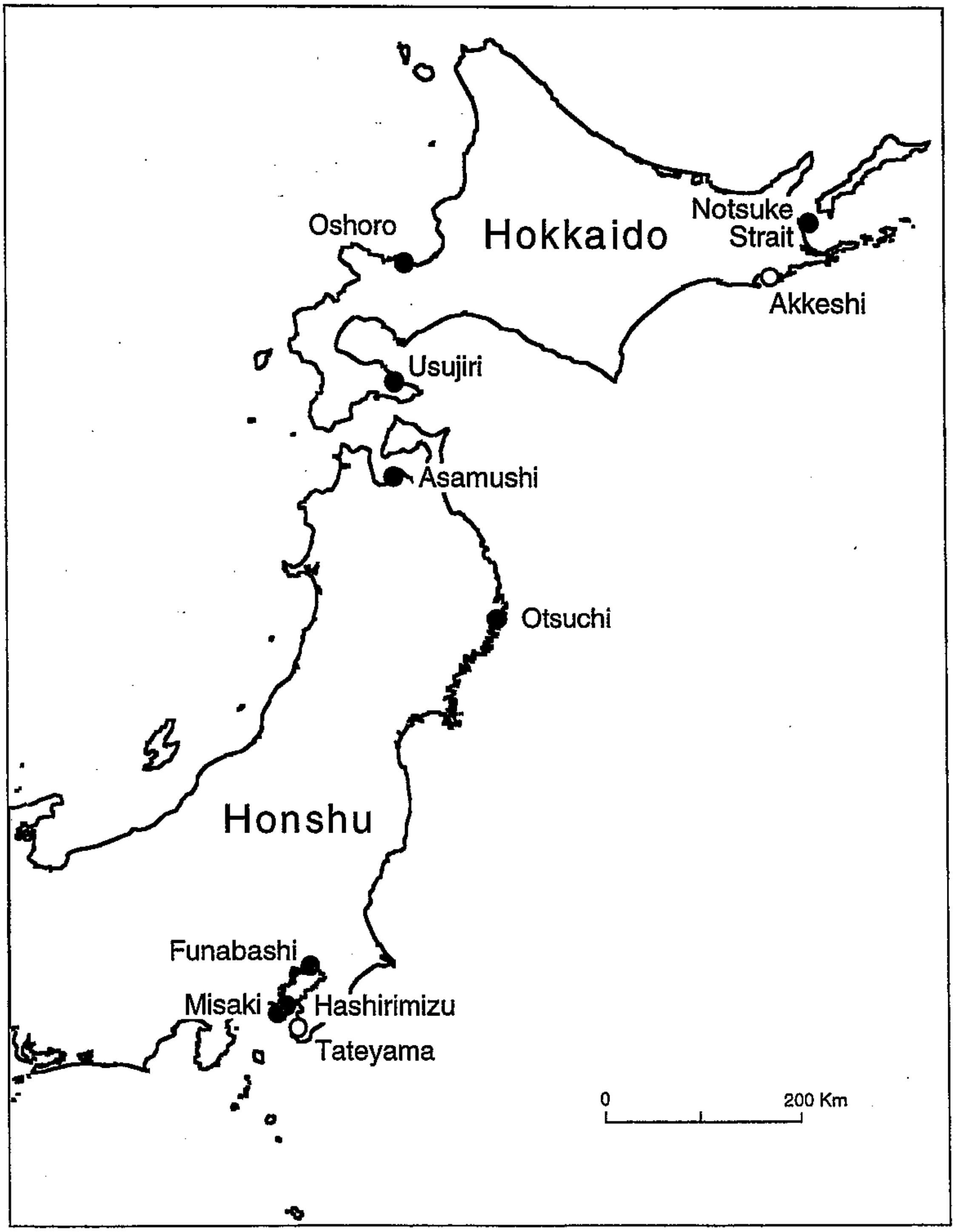

Figure 1. Map of northern Honshu and Hokkaido Islands, Japan, showing locations where *Asterias amurensis* were collected for parasitological investigations (●). Marine stations where seastars were examined but not locally collected are denoted by O.

RESULTS

PARASITOLOGICAL INVESTIGATION OF *A. AMURENSIS*

Table 1 summarises the localities, collecting methods, mean sizes and colours of collected seastars, the number of seastars examined by the digestive gland squash technique. Figure 1 locates the collecting sites on a map of northern Honshu and Hokkaido. A total of 489 seastars was examined for parasites.

Size was highly variable. Although differences in collecting methods certainly accounted for some of the differences between populations, it is clear that there are substantial differences between populations and a trend for more northern locations to have larger seastars than the sites in central Honshu. It is notable that at some sites (at Otsuchi) the mean size of seastars was larger than the maximum size of seastars reported from Tasmania waters (Morrice 1995).

Colour was variable and again showed some geographic patterns. The seastars at the southernmost locations were uniformly yellow. Further north, some sites were variable, some predominately purple, and a few predominantly yellow. Although the basis for variation in colour of *A. amurensis* is unknown, observations on a purple individual regenerating yellow arms suggest that colour is not genetically fixed and can change over the life of an individual.

Table 2 reports the data on gonadal squash examinations. Since *A. amurensis* spawns in the spring, our July samples were post-spawning and in most cases sex could not be determined because the gonads were very small and no gametes were present. Gonads were not macroscopically detectable in 27% of the dissected seastars and no gametes were present in a further 63%. Where seastars could be sexed it is of interest that our samples tended to be either predominately male or female, suggesting spatial separation of the sexes in these populations. This was confirmed by discussions with some of the Japanese developmental biologists at our host laboratories.

No dendrogastrid ascothoracidans were recovered. This was not unexpected based on the lack of such reports in the literature. We also did not observe any eulimids.

A single male seastar from Nemuro in the Notsuke Strait (separating Hokkaido from the Kurile Islands) was infected with the parasitic ciliate, *Orchitophrya stellarum* (Table 2). Prevalence of this parasite was 2.6% of all examined starfish from Nemuro, 2.8% of all seastars from Nemuro whose gonads were examined, and 6.2% of male starfish from Nemuro. Overall prevalence was 0.3% of all seastars whose gonads were examined and 1.2% of all males examined. Compared to published descriptions (reviewed by Jangoux 1987), the infection in the parasitised seastars was of low to moderate intensity. Active sperm were relatively numerous and no ciliates were present in coelomic fluid samples.

Table 1. The locations, sites, total number of *Asterias amurensis* examined by all methods, number of seastars examined by alcohol washing only, mean size (longest arm length), size, range, color (percentage yellow includes seastars that were yellow plus those that were predominately yellow (yellow/purple)), and the number of seastars examined by the digestive gland squash method.

Location Site	Number examined	Number washed	Size mean	Size (range)	Colour % yellow	Digestive gl. squashes	Depth (m)	Collection method	Comments
Funabashi									
Gyotoku	3	–	26	(22–23)	100	–	intertidal	by hand	anoxic,polluted water
Tateyama									
Miura-Hashirimizu Mabori Kaigan	32	–	72	(63–91)	100	32	intertidal	by hand	specimens held several months
Misaki									
Miura-Hashirimizu Hashirimizu Port	26	–	75	(56–108)	100	26	3–4	rake	
Misaki-Aburatsubo	41	–	89	(62–109)	100	41	3–4	rake	off Misaki MBL
Otsuchi									
Murohama	20	–	146	(98–225)	28	20	3–4	scuba	at fishing port (17°C)
Off Murohama	20	–	213	(130–227)	5	20	11–14	scuba	under scallop aquaculture, mud (15°C)
Hakozaki	20	–	107	(63–145)	<10	20	2–3	scuba	fishing port; mud (18°C)
Hakozaki-Shirahama	20	–	106	(70–140)	5	20	2–3	scuba	fishing port; sand/mud (18°C)
Asamushi									
Futagojima	24	4	90	(38–147)	0	20	20–25	trap	
Tsuchiya	24	4	91	(57–152)	0	20	9–11	trap	
Oura	24	4	86[a]	(49–143)	0	20	12	scuba	
Hadakajima	36	16	74[b]	(40–124)	0	20	intertidal–5	hand, snorkel, scuba	12 specimens individually bagged
Moura	24	4	83	(51–123)	5	20	3–5	trap	

(continued on next page)

Table 1 continued

Location Site	Number examined	Number washed	Size mean	 (range)	Colour % yellow	Digestive gl. squashes	Depth (m)	Collection method	Comments
Akkeshi									
Shibetsu	6	2	130	(97–182)	83	6	?	scallop dredges	held in poor conditions; remainder were dead
Nemuro	43	4	117	(80–190)	95	20	?	scallop dredges	
Notsuke	39	4	112	(46–205)	67	20	?	scallop dredges	
Oshoro									
S. Shore	32	10	76	(57–101)	0	20	0–2	snorkel	10 specimens individually bagged
Usujiri									
Usujiri–deep	25	4	83	(46–160)	8	20	30	trap	sand
Usujiri–shallow	30	4	125	(73–153)	17	20	5–10	trap	rocky
Total seastars	489	60				385			

a N = 20
b N = 35

Table 2. Summary of gonadal examinations of *Asterias amurensis*. Unsexed specimens had gonads but no gametes were present. No gonads refers to seastars with gonads too small to be detected or excised for microscopic examination. Ciliate prevalence is the percentage of seastars examined at each site that were infected with *Orchitophrya stellarum*.

Location / Site	Male	Female	Unsexed	No gonads	Ciliate prevalence (%)
Funabashi					
Gyotoku	–	–	–	–	–
Tateyama					
Miura-Hashirimizu Mabori Kaigan	0	4	23	5	0
Misaki					
Miura-Hashirimizu Hashirimizu Port	6	0	17	3	0
Misaki-Aburatsubo	0	0	34	7	0
Otsuchi					
Murohama	5	1	12	3	0
Off Murohama	4	11	5	0	0
Hakozaki	9	2	9	0	0
Hakozaki-Shirahama	6	1	12	1	0
Asamushi					
Futagojima	1	0	14	5	0
Tsuchiya	3	0	8	9	0
Oura	1	0	7	12	0
Hadakajima	0	0	10	10	0
Moura	0	0	6	14	0
Akkeshi					
Shibetsu	2	1	2	1	0
Nemuro	16	15	5	7	3
Notsuke	1	9	24	5	0
Oshoro					
S. Shore	8	0	16	8	0
Usujiri					
Usujiri-deep	5	0	9	11	0
Usujiri-shallow	15	0	10	5	0
Total seastars	83	44	223	103	

Concerned about the possibility of having missed other infections, we repeated our squash procedure with a further 5 pieces of gonadal tissue from that seastar. Ciliates were very readily detected in all 6 samples. Thus, our sampling procedure appeared to be highly sensitive to detection of ciliate presence. Detection of these ciliates will undoubtedly be easier in the spring when gonads are enlarged and filled with gametogenic cells.

No *Scottomyzon gibberum* were observed in the central Japan samples (Table 3). However, quantitative comparisons of visual searches with alcohol-washed specimens suggest that visual samples are not an efficient means to detect the presence of copepods. From Mutsu Bay north, *S. gibberum* was usually present. Although the data on abundance and intensity were variable, it may have been more abundant in the deeper samples (> 10m) and in colder water (Notsuki Strait).

In his monograph on the biology of these copepods, Rottger (1969) divided development of mature females into 6 stages, based on the expansion of the inflated head and thorax. In this developmental sequence stage 1 was described as copepodid V, while stages 2–6 were subdivisions of the adult instar in which the head and thorax progressively expand without intervening moults. Early stages are relatively unmodified and are active ectoparasites moving about the surface of their host. In the later stages the copepod becomes attached to the host and is enveloped in a cyst formed from hypertrophied tissue at the base of pedicellaria and sometimes spines on the aboral surface. All our adult females were in the early active stages (1 or 2) and often bore egg sacs. In contrast, Rottger's specimens did not bear egg sacs until stage four, when the females are effectively attached. Rottger, working on copepods from the Atlantic *A. rubens*, noted that cyst formation was strongly seasonal, developing in the fall. Presuming a seasonal development of *S. gibberum* on *A. amurensis*, it would be necessary to re-sample these copepods in the fall to quantify associated pathology.

Table 3 also records the presence of a scaleworm, *Arctonoe vittata*, on the aboral surface. These large epibionts were not seen at the central Japan sites and were relatively uncommon at most northern sites. Polynoid polychaetes associated with other seastars are generally considered commensals and there is some evidence that their relationship may even be mutualistic (Dimock & Dimock 1969, Wagner *et al.* 1979). Britayev (1991) reviews the association between this scaleworm and *Asterias amurensis*.

Washed samples recovered a few specimens of several types of crustaceans (amphipods, isopods, copepods) and turbellarians. All may have been incidental to the use of raw sea water in the washing process although turbellarians and caprellids have been reported as seastar epibionts, sometimes in abundance (Rottger 1969).

There was no evidence of infectious disease in any examined lesion. A few specimens exhibited iridescent discoloured tube feet or necrotic lesions, often in animals held for a long time in captivity (e.g., at Tateyama).

Table 3. Summary of the examination of *Asterias amurensis* for ectosymbionts, particularly the copepod *Scottomyzon gibberum* and the polynoid polychaete scaleworm, *Arctonoe vittata*. Prevalence is percentage infested, abundance (A) is the mean number of symbionts on all seastars examined in a pooled sample of four seastars and intensity (I) is the mean number recovered from infested seastars only (individually washed). (–) indicates that intensity could not be calculated since no seastars were infested; (+) indicates that copepods were present but seastars were in poor condition and quantification was not attempted.

Location Site	Number Examined	*S. gibberum* Prevalence	*S. gibberum* Intensity/ Abundance	*A. vittata* Prevalence	*A. vittata* Intensity/ Abundance
Funabashi					
Gyotoku	3	0	–	0	–
Tateyama					
Miura-Hashirimizu Mabori Kaigan	32	0	–	0	–
Misaki					
Miura-Hashirimizu Hashirimizu Port	26	0	–	0	–
Misaki-Aburatsubo	41	0	–	0	–
Otsuchi					
Murohama	20	0	–	17[a]	I=1
Off Murohama	20	0	–	0[a]	–
Hakozaki	20	0	–	0	–
Hakozaki-Shirahama	20	0	–	0	–
Asamushi					
Futagojima	20	35	I=13	0[b]	–
Tsuchiya	20	40	A=12	0	–
Oura	20	10	A=6	30	I=1 A=0.5
Hadakajima	12	0	A=20	42[c]	I=1.2
Moura	20	45	A=7	0	A=0
Akkeshi					
Shibetsu	6	+	–	0	–
Nemuro	20	5	A=14	5	I=1
Notsuke	20	20	A=20	0	–
Oshoro					
S. Shore	20	15	I=1	5	I=1
Usujiri					
Usujiri-deep	21	0	A=6	0	A=1.2
Usujiri-shallow	20	0	–	20	I=15
Total seastars	381				

a, N = 28

b, N = 24

c, N = 12

INVESTIGATION OF OTHER SPECIES OF SEASTARS

Only a few other species of starfish were obtained and there was little time available to dissect them. No dendrogastrids, *Orchitophrya* or *Scottomyzon* were recovered. *Asterina pectinifera* was common from Asamushi north. One of us (MJG) had previously examined large numbers of this species for dendrogastrids without recovering any so this species was not dissected. *Distolasterias nipon* was obtained at Asamushi, Akkeshi and Usujiri. Six were dissected and one was alcohol washed. One scaleworm was recovered. Although no *Scottomyzon* were obtained, 57 specimens of other species of copepods were collected. *Aphelasterias japonica* was observed in the intertidal zone and shallow subtidal areas at Asamushi, Oshoro and Usujiri. Four were dissected.

DISCUSSION

PRELIMINARY EVALUATION OF CONTROL AGENTS

The ciliate, *Orchitophrya stellarum*, merits some consideration as a potential control agent because it has a direct and substantial effect on male gonads, feeding on the germ cells (Bouland & Jangoux 1988), has repeatedly been associated with low male sex ratios, mortality of males (particularly in large size classes) and reduced recruitment following years of high infection levels (Vevers 1951, Leighton, *et al.* 1991, Claereboudt & Bouland 1994, Jangoux 1987). Several theoretical issues immediately arise. What is the role of sperm limitation in the larval abundance of seastars? Can reductions in male sperm output significantly effect fertilisation rates? This is an active area at the interface between developmental biology and marine ecology.

Logic and available evidence suggests that fertilisation rates decline with decreasing sperm abundance and distance from males (Levitan 1991, Vogel *et al.* 1982, Denny & Shibata 1989). Clearly, in order to model the effect of *O. stellarum* on recruitment of *A. amurensis* it will also be necessary to know a good deal more about sex ratios, impact on male survivorship, recovery rates (Bang 1982 claimed rapid recovery, 15 days, from *O. stellarum* in *A. rubens*), spawning behaviour (synchronicity, aggregation responses), size-specific gamete output and infestation rates, and effects on female hosts. Why do some studies report high prevalence in female seastars but others report prevalences approaching nil in females (see Jangoux 1987, 1990)? Even if the effect of the ciliate is limited to castrating males, there is mounting evidence that the fertilisation of ova is limited by sperm availability in broadcast spawning invertebrates (Levitan & Petersen 1995). If this is true for *A. amurensis* (and we assume that it should be), *O. stellarum* could reduce seastar population densities (at some spatial scales) if it reached sufficiently high prevalence.

The nominal species, *O. stellarum*, has been reported from several geographic regions and seastar species with little in the way of comparative studies. It will be necessary to establish host specificity of this probable cluster of species or strains. If the *O. stellarum* from *A. amurensis* is indeed conspecific with the often highly prevalent and well-studied ciliates of *A. rubens*, it may be desirable to obtain ciliates for further study from the latter more accessible species. Although our study establishes a new host record for *O. stellarum*, it provides no insight into its geographic distribution in Japan. Is *O. stellarum* present only (or at higher frequencies) in Notsuke Strait, or is it merely rare, but widespread, in Japan? This is an important logistic issue because the Akkeshi Marine Biological Station, which obtained these seastar from local fishermen, is the most remote locality we visited, about 6 hours by train from Sapporo. Winters there are severe and ice scours the intertidal zone every 3-4 years (K. Sano, pers. comm.). It would be a challenging site for extensive studies in residence.

The copepod *Scottomyzon gibberum*, although recorded from *A. amurensis* by Kim (1992), is less studied. Issues of species identification and host specificity are also pertinent here. Comparisons with Rottger's (1969) monographic study of this copepod in the North Sea suggest such substantial life history differences that the conspecific identity of the copepods from A. *amurensis* is problematic. It's potential as a possible natural enemy for biological control seems less than that of the ciliate. For a complete determination, it will be necessary to establish the relationship between intensity and pathology and to determine if the lesions induced by encysted females become necrotic (if indeed females encyst on *A. amurensis*), and what effect such damage has on seastar survivorship.

Both the ciliate, *O. stellarum,* and the copepod, *S. gibberum,* are relatively cryptic. We suggest that Tasmanian populations of *A. amurensis* be surveyed for copepods and ciliates by alcohol washing and gonadal squash techniques respectively, since it is possible that these parasites, or similar related species, may have transferred from the native asteroid fauna to *A. amurensis*. If not, their absence will serve as preliminary evidence for unrecognised host specificity.

The search for dendrogastrids should be concluded. None were previously reported despite the relatively considerable work on this group in Japan by Okada, Grygier and others. Further, no such parasites have ever been reported from any species of *Asterias*. However, some consideration might be given to the native *Dendrogaster tasmaniensis*, known from *Allostichaster polyplax* (Hickman 1959). Perhaps it is capable of parasitising *Asterias amurensis* under laboratory conditions.

Beyond the opportunity now presented to further examine *O. stellarum* or *S. gibberum* as potential natural enemies there is one other possible avenue of investigation. The outbreaks of *A. amurensis* (briefly reviewed in Hawkes & Day 1993, Morrice 1995) produce populations of very high densities that apparently soon subside. These outbreaks apparently are more likely to reoccur at certain locations. These highly successful recruitment events have received some investigation but their declines have been little studied. We note that a principal pre-condition for an infectious disease to reach epidemic proportions is high host population density. Locations with seastar populations two or three years after a seastar outbreak are the most likely places to yield infectious agents that may be suitable for control in Tasmania. Since these outbreaks are infrequent, localised and somewhat unpredictable, this investigation will require an expansion of contact with Japanese biologists to gather news of abundance of appropriate Japanese seastar populations.

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