

NOTE

Castration and mortality in *Pisaster ochraceus* parasitized by *Orchitophrya stellarum* (Ciliophora)B. J. Leighton¹, J. D. G. Boom¹, C. Bouland², E. B. Hartwick¹, M. J. Smith¹¹ Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6² Department of Biology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2

ABSTRACT: A survey of the sex-ratio and gonadal index of sea stars *Pisaster ochraceus* (Brandt, 1835) at 2 sites on the southern coast of British Columbia, Canada, revealed an epizootic disease affecting the testes. The disease reduced the proportion of males and their reproductive potential in the populations studied. Examination of diseased testes revealed the presence of a parasitic ciliate, *Orchitophrya stellarum* Cépède, 1907, that has not previously been reported from the Pacific Ocean. The more virulent nature of the parasite in *P. ochraceus*, as compared with its Atlantic and Mediterranean hosts, may indicate the recent introduction of the parasite into the Pacific.

The only ciliate known to parasitize echinoderms is the scuticociliate *Orchitophrya stellarum* (Cépède 1907a, b, Bouland et al. 1987). *O. stellarum* has been reported several times from asteroid testes and occasionally from asteroid ovaries (for review, see Jangoux 1987). Its presence has only been reported in the North Atlantic Ocean and the Mediterranean Sea. Although the parasite does not appear to damage the ovary, infestation of males results in testicular regression and partial castration (Cépède 1907a, b, 1910). Characteristics of pathological changes in infested *Asterias rubens* include regression in size and a peculiar violet to blueish colouration of the testes, partial to complete disorganization of the germinal epithelium (Bouland & Jangoux 1988) and cloudy coelomic fluid (Taylor & Bang 1978). According to Bang (1982), infested individuals recover from infection. No mortality has been associated with the presence of the parasite in Atlantic or Mediterranean sea stars.

Boom (1988) reported diseased testes and a reduced proportion of males in populations of *Pisaster ochraceus* on the southern coast of British Columbia, Canada. Here we report on this gonad disease and its

consequences in *P. ochraceus* and the occurrence of the ciliate *Orchitophrya stellarum* in a Pacific sea star.

Methods and materials. Specimens of *Pisaster ochraceus* were collected by SCUBA at a depth of 0 to 10 m in Indian Arm during the years 1980, 1981, 1985 and 1987 to 1989, and from Bowen Island during 1981 and 1987 to 1989, both sites located near Vancouver, British Columbia. The sex of the sea stars was determined by examining a fresh gonad smear under a standard compound microscope (magnification = 100×) and the sex-ratio of males to females was recorded. Gonadal index (gonad wet weight/wet weight of whole individual) was recorded in April and May 1985, 1987, and 1989 and the data were pooled for the 2 sites.

In spring 1989, 60 *Pisaster ochraceus* were collected from the Bowen Island site to study the disease. The external condition and sex-ratio of these sea stars were recorded and the gonads were examined in Millipore-filtered seawater under a dissecting microscope. The coelom and coelomic fluid were also examined. In order to identify the ciliate, samples of diseased gonads were fixed and stained for light microscopy and prepared for scanning electron microscopy according to Bouland et al. (1987). The statistical methods (chi-square and Student's t-test) were used according to Zar (1984).

Results and discussion. The sex-ratios from 1980 and 1981 were not significantly different from 1:1 (chi-square, $p > 0.05$; Table 1). In 1987, 1988 and 1989 the sex-ratios were lower and significantly different from 1:1 ($p < 0.001$). The proportion of males in the sampled populations had been reduced by more than 80% between 1981 and 1987. We have assumed that a 1:1

Table 1. *Pisaster ochraceus*. Sex-ratio of specimens from Bowen Island (BI) and Indian Arm (IA), Vancouver, British Columbia. Each sex-ratio is tested against a 1:1 ratio

Site	Year	n	Sex-ratio M/F	1:1 ratio	Chi-square (df = 1)	Probability (p)
IA	1980	20	1.22	Yes	0.20	NS ^a
IA	1981	108	1.46	Yes	3.70	NS
BI	1981	89	1.41	Yes	2.53	NS
IA	1987	150	0.15	No	80.67	(p < 0.001)
BI	1987	41	0.14	No	23.44	(p < 0.001)
BI	1988	92	0.17	No	47.35	(p < 0.001)
IA	1989	61	0.17	No	30.31	(p < 0.001)
BI	1989	80	0.25	No	28.80	(p < 0.001)

^a Probabilities greater than 0.05 were considered non-significant (NS)

ratio is the normal condition in *Pisaster ochraceus*, as both Feder (1957) and Menge (1975) indicated 1:1 sex-ratios in populations of *P. ochraceus* on the California (USA) coast and in the San Juan Islands (Washington, USA), respectively.

In April and May, 1987 and 1989, the male gonadal indices were significantly lower than in April and May 1985 (t-test, $p < 0.001$; Table 2). The male gonadal index was also significantly lower than the female gonadal index in 1989 ($p < 0.001$). The female gonadal index in 1989 (14.70%) was not significantly different ($p < 0.05$) from the male index in 1985 and did not differ greatly from the peak gonadal indices (13 to 16%) reported by Feder (1957) or Nimitz (1971, 1976). These data were taken in April and May when gonadal indices in *Pisaster ochraceus* are normally at their pre-spawning peaks (Feder 1957, Farmanfarmaian et al. 1958, Mauzey 1966, Nimitz 1976, Fraser et al. 1981). Farmanfarmaian et al. reported no significant difference between male and female gonadal indices. The abnormally low gonadal indices of male *P. ochraceus* during this period in 1987 and 1989 are coincident with a pathological state affecting only the male sea stars.

The first observations of discoloured male testes were recorded in 1986 (see Boom 1988). Evidence that an epizootic was occurring was first noted in 1987

when male gonad disease became prevalent with a reduction in the frequency of male sea stars in the samples. The unusual occurrences of dead *Pisaster ochraceus* at the collecting sites in spring 1987 and 1988 were recorded by divers that had visited sites on a monthly basis since 1978.

Of 60 *Pisaster ochraceus* collected from Bowen Island in spring 1989, 11 were males (sex-ratio = 0.18) and 4 (36.4%) of these males exhibited gonad disease in the form of flaccid, tan-coloured testes instead of the creamy white appearance of healthy mature male gonads. These signs appear most often at the distal tip of the testis but occasionally the entire gonad is reduced in size and discoloured. The diseased sea stars in this sample showed some evidence of infection in all 10 of their testes.

Ciliates were found in the testes of 3 of the 4 diseased sea stars and in none of the other males or females. These ciliates were identified as *Orchitophrya stellarum* and were indistinguishable from the Atlantic species, which castrates testes in sea stars in the Atlantic Ocean and the Mediterranean Sea (Jangoux 1987). This is the first record of the ciliate in the testes of a Pacific sea star.

The 2 most heavily infested individuals were moribund and externally discoloured with cloudy

Table 2. *Pisaster ochraceus*. Gonadal indices. The 1987 and 1989 data were tested against the 1985 data. Data were pooled from the 2 collecting sites

Date	Sex	n	Mean (%)	Standard deviation	t ^a	Probability (p)
Apr–May 1985	M	15	15.42	3.89		
Apr–May 1987	M	19	3.68	2.32	-22.15	(p < 0.001)
Apr–May 1989	M	11	3.00	1.67	-24.84	(p < 0.001)
Apr–May 1989	F	29	14.70	6.98	0.55	NS ^b

^a Student's t-test
^b Probabilities greater than 0.05 were considered non-significant (NS)

coelomic fluid. The other 2 diseased males appeared healthy externally but the coelomic fluid was cloudy. Although some sperm was present in the coelomic fluid, the cloudiness was due to large numbers of coelomocytes and cell debris in the fluid. All other sea stars appeared healthy externally and had relatively clear coelomic fluid.

In the diseased male in which ciliates were not found, the gonads were totally shrivelled and discoloured. This individual may represent a host which has survived castration and has cleared itself of the parasite. With the exception of the colour of the diseased testes, the gross pathological changes of the gonads of *Pisaster ochraceus* are similar to those reported in *Asterias rubens* (Boulard & Jangoux 1988).

There are no reports of host mortality or external morbidity of Atlantic or Mediterranean hosts of *Orchitophrya stellarum*; *Asterias forbesi* from the northwest Atlantic has been found to recover from the infection within 10 to 15 d in the laboratory (Bang 1982). The external discolouration and morbidity observed in heavily infected *Pisaster ochraceus* and the presence of dead sea stars of this species at the collecting sites suggest that a lethal epizootic is in progress in these populations. The large reduction in the proportion of males in the populations implicates a sex-specific disease agent such as *O. stellarum*. The greater virulence that *O. stellarum* has demonstrated in *P. ochraceus* compared to its Atlantic hosts, and the epizootic nature of the disease at the study sites, may indicate the recent introduction of this parasite to the Pacific coast.

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