

Seasonal visceral atrophy and response to salinity by *Parastichopus californicus* (Stimpson): Osmoregulation?

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Introduction

Autonomy in response to physical or physiological stress is characteristic of many echinoderm species. Expressions of this phenomenon include brachial loss (sea stars and brittle stars) and ejection of the viscera in sea cucumbers. Although evisceration by sea cucumbers has rarely been observed *in situ*, it is a response commonly evoked by holothuroids mistakenly hooked by fisherman, following rough collection, being husbanded in stagnant sea water, exposed to elevated temperatures, or artificially traumatised in the laboratory via electric shock, mechanical pinching, injections of ammonium hydroxide, or strychnine (see Byrne 2001).

Numerous anecdotal observations of evisceration by holothurians *in vitro* have led to the premature conclusion that sea cucumbers also eviscerate in their natural environment, and may do so as a natural response to predation. Presumably, ejected viscera function as a decoy to occupy a predator while the sea cucumber makes an escape (Mottet 1976; Pearse et al. 1987). Direct observations of evisceration occurring in nature are singular. A study by Byrne (1985) reports on seasonal evisceration behaviour occurring *in situ* by the dendrochirotid sea cucumber *Eupentacta quinquesemita*. Seasonal evisceration has been concluded to occur (in the absence of direct observation) for *Actinopyga agassizi* (Mosher 1965), *Parastichopus californicus* (Swan 1961), *Stichopus regalis* (Bertolini 1932), and *Stichopus tremulus* (Jerpersen and Lutzen 1971; Lutzen 1979).

Swan (1961) noted that of the 81 specimens of *Parastichopus californicus* he collected during autumn at Friday Harbor, Washington USA, 49 individuals possessed incomplete visceral organs. During the winter months, however, all of the 70 specimens he examined contained normal and complete viscera. Thus, Swan concluded that *P. californicus* had undergone spontaneous seasonal evisceration. However, when the visceral condition in a population of *P. californicus* was examined monthly over a period of three and more years

(Fankboner and Cameron 1985), it was discovered that the gut, respiratory tree, circulatory system and gonad were annually lost as a result of atrophy of these organs (Figs. 1 and 2), and not, as Swan (1961) had concluded, the outcome of spontaneous, seasonal evisceration. Accompanying atrophy of the visceral organs is the expulsion of visceral particulates, parasitic gastropods, parasitic worms, their eggs and parasitic protozoans (gregarines) through hundreds of transrectal (AKA perianal) coelomoducts connecting the coelom with the sea cucumber's cloacal chamber (Fankboner and Cameron 1985; Shinn 1985; Shinn et al. 1990). Atrophied viscera of *P. californicus* regenerate within several weeks following clearance of the coelomic cavity. Metabolites — arising during the atrophy process from the viscera and the body wall — sustain the sea cucumber in the absence of feeding. The latter experiences a 25 per cent reduction in mass during loss and regeneration of the viscera (Fankboner and Cameron 1985). Seasonal shrinking of individual visceral organs has also been reported in *Stichopus japonicus* (Choe 1963; Tanaka 1958; Suguri 1965).

Clearly there are two beneficiaries from the occurrence of seasonal visceral atrophy. Symbionts living within the coelom of the sea cucumber benefit evolutionarily when they (and their eggs) are evicted from the coelom, allowing for new generations of their species to infect *P. californicus*. Parasitic eugregarine protozoans, the umagillid turbellarians *Ozametra* sp. and *Anoplodium hymanae* live within the coelom of *P. californicus* and consume the sea cucumber's intestine and coelomocytes, respectively (Shinn 1985). It is evident that the free-floating eggs of the parasitic gastropod *Enteroxenos bonnievie* (Lutzen 1979) are released from *P. californicus* through these same ducts. Thus, seasonal visceral atrophy provides the host, *P. californicus*, an opportunity and means to replace viscera damaged by coelomic parasites. Although seasonal visceral atrophy occurs over a brief period in the fall, this process may also improve the resistance by *P. californicus* to environmental salinity extremes created by seasonal increases in precipitation. The experiments

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described in this article, test the hypothesis that seasonal visceral atrophy in *P. californicus* reduces the surface area of its tissues exposed to osmotic stress, and by so doing, may improve its resistance to short exposures to autumnal lows in salinity.

Methods

Forty-five adult specimens of *P. californicus* were collected in late August using scuba at depths between 6 m and 10 m adjacent to



Figure 1. *P. californicus* which has ceased tentacular feeding/locomoting and has become soporific. This sea cucumber is undergoing the process of seasonal visceral atrophy.

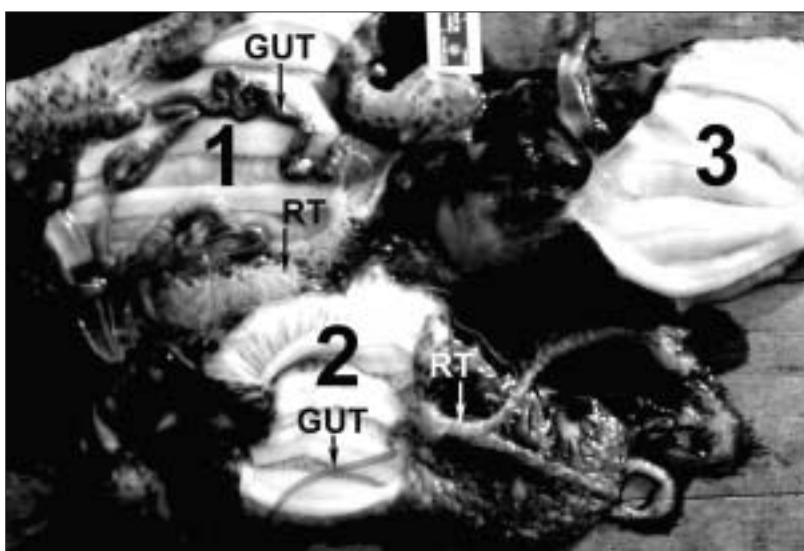


Figure 2.

Three specimens of *P. californicus* that have been dissected to reveal the condition of their visceral organs.

- 1: *P. californicus* which has normal viscera. The gut (GUT) is filled with food; respiratory trees (RT) complete.
- 2: The empty gut (GUT) in the specimen of *P. californicus* has become shrunken, and brittle in the course of the early phase of seasonal visceral atrophy; the respiratory trees (RT) in this preparation have been partially resorbed.
- 3: Completed visceral atrophy in *P. californicus*.

Croker Island, Indian Arm Fjord, British Columbia (49°20'N, 122°55'W). The sea cucumbers were quickly replaced in saltwater aquaria at Simon Fraser University and acclimatised for one week at a salinity of 25‰ and a constant temperature of 12°C. None of the experimental *P. californicus* fed during the periods of acclimation and the experiments.

Experimental animals were sorted into five replicate groups with each group containing nine specimens similar in size range to the other four groups. At the beginning of each of five experimental series, nine sea cucumbers were individually labelled by anchoring a numbered spaghetti tag (Flow Tag Inc., 4616 Union Bay Place NE, Seattle, Washington USA 98105) through each sea cucumber's body wall. By so doing, the progress of individual sea cucumbers could be followed at 1.5-hour periods throughout the six hours of coelomic fluid sampling. Earlier testing of spaghetti tags in *P. californicus* at Friday Harbor Laboratories, Washington State, USA, indicated that their presence had no significant influence the sea cucumber's behaviour nor did they affect its ability to react to shifts in the salinity of the experimental medium.

Each replicate of nine sea cucumbers was placed in a separate 250-litre aquarium and processed on sequential and separate days. At the beginning of each test series, dechlorinated fresh water was added to resident sea water in the aquarium until the salinity was lowered from the *in situ* salinity of 25‰ (mixing time ≈ 5 minutes) to sea water representing the minimum salinity 15‰. *P. californicus* might experience during periods of fresh water winter runoff. Next, the volume of sea water in the experimental aquarium was adjusted to equal to its original 250 litres.

Two to three drops of coelomic fluid were extracted from each sea cucumber at 0.0 hours, 1.5 hours, 3.0 hours, 4.5 hours, and 6.0 hours using separate 1.0 ml disposable TB syringes and No. 21 gauge 1.5" needles. Extracts of coelomic fluid were kept ice cold within the syringes and analysed within 15 minutes to

reduce potential errors in measurement of osmotic pressure, which might result from degeneration of the samples. A 10 μ l subsample was taken from each syringe and analysed in a Wescor Model 5100B Vapor Pressure Osmometer. Typically, osmotic determinations took less than 1.5 minutes.

The osmotic pressure data for coelomic fluid sampled from each sea cucumber was placed into one of two groups based on the presence or absence (atrophy) of visceral organs in the sea cucumbers. Within the two separate groups, sample data were pooled for individual time periods. After applying light pressure on the body wall to clear the rectal lumen and/or the respiratory trees of sea water, each experimental *P. californicus* was weighed at 0.0 hours and 6.0 hours to ascertain whether the sea cucumbers might experience volume changes of coelomic fluid in response to osmotic stress.

Results

The osmotic pressure of the coelomic fluid from *P. californicus* (Fig. 3) with intact viscera fell steadily throughout the six-hour experimental period from 701.23 mOsmol/kg to 581.50 mOsmol/kg. Coelomic fluid from *P. californicus*, which lacked visceral organs (Fig. 4), followed a similar decline in osmotic pressure for the first three hours of the experiment, but rose dramatically to a level nearly that of pre-experimental values by 4.5 hours. Coelomic fluid osmolality in *P. californicus* lacking viscera appeared to stabilise at between 4.5 and 6.0 hours, and at the termination of the experiments had attained a value that was 90 mOsmol/kg higher than sea cucumbers with intact viscera. This recovery of osmotic pressure in the coelomic fluid of visceraally atrophied *P. californicus* to nearly pre-experimental levels suggests that these sea cucumbers possess at least a limited ability to osmoregulate against a declining salinity gradient.

During the six-hour experimental period, body weights increased in both visceraally atrophied (15.3%) and visceraally intact sea cucumbers

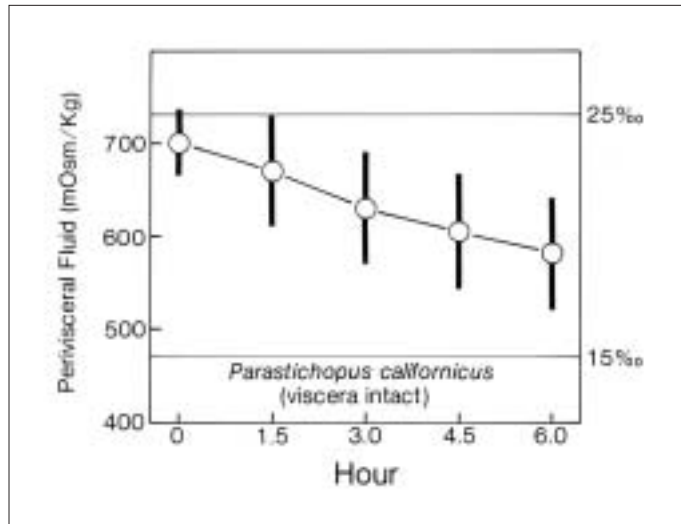


Figure 3. Changes in the osmotic pressure of coelomic fluid of *P. californicus* with intact viscera when transferred for six hours from a salinity of 25‰ to 15‰.

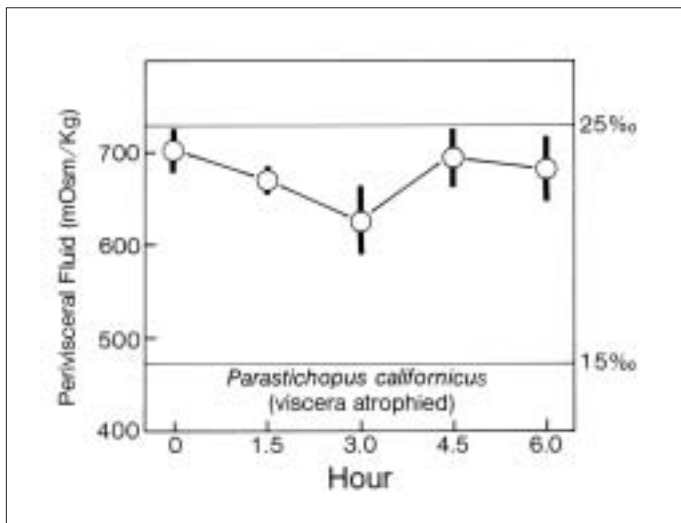


Figure 4. Changes in the osmotic pressure of coelomic fluid of *P. californicus* with atrophied viscera when transferred for six hours from a salinity of 25‰ to 15‰.

(29.5%) indicating that volumetric increases occurred in *P. californicus* during the course of the experiments. *P. californicus* with intact viscera increased its body weight during the experimental period by 29.5%, a level of increase that was effectively twice that found in visceraally atrophied sea cucumbers. No doubt this difference could be explained by the presence of viscera in the former, which provided additional pathways (the respiratory trees and the intestine) for osmotic transport of hyposaline water to the coelom than in visceraally atrophied sea cucumbers.

Discussion

The phylum Echinodermata has been characterised by the absence of discrete nephridial organs (Ruppert and Barnes 1994; Hyman 1955). Indeed, it is the inability of echinoderms to osmoregulate (Binyon 1972) that may have inhibited members of this extensive deuterostome phylum (6000 species extant) from invading fresh water and terrestrial environments. There is, however, some evidence that indicates that at least limited resistance to sudden changes in environmental salinity occurs in some echinoderms (Choe 1963; Freeman 1966; Giese and Farmanfarmanian 1963; Pearse 1967; Stickle and Denoux 1976; Stickle and Diehl 1987; Turner and Meyer 1980; Kashenko 2002). In addition, the discovery of a podocyte-lined channel connecting the axocoel to an external pore in both the bipinnaria larvae of the sea star *Asterias forbesi* (Ruppert and Balser 1986), and the auricularia larvae of *Holothuria grisea* (Balser and Ruppert 1993) suggest that organs for osmoregulation could be present in some adult echinoderms.

The results of the experiments described in this paper suggest that coelomic fluid from visceraally atrophied specimens of *P. californicus* loses osmotic pressure for the first few hours at the same rate as *P. californicus* with intact visceral organs. After hour 3, the coelomic fluid of *P. californicus* with intact viscera continues its downward trend, while in contrast, the coelomic fluid in visceraally atrophied *P. californicus* recovers and stabilises to about its pre-experimental level. These data suggest that visceraally atrophied *P. californicus* possess at least a limited capacity for osmoregulation against a lowering in salinity. In visceraally intact *P. californicus*, 15‰ sea water contacting the high surface areas of the respiratory trees and the intestine diffuses easily into the sea cucumber's perivisceral coelom. This incursion of sea water progressively dilutes the 25‰ coelomic fluid until it establishes a 90 mOsmol/kg mean differential with the osmotically stabilised coelomic fluid of visceraally atrophied animals.

There are two pathways for osmotic regulation of the coelomic fluid of visceraally atrophied *P. californicus*. First, the formation of additional particulates in the coelomic fluid would attenuate the effects of brackish water influx, and could maintain the osmotic pressure at pre-experimental levels. There is no evidence, however, to suggest that such an increase in coelomic fluid particulates has occurred in these experiments. A second proposed pathway for visceraally atrophied *P. californicus* to regulate the osmotic pressure of its coelomic fluid would include removal of lower salinity water from the coelomic fluid and ejecting it to the outside

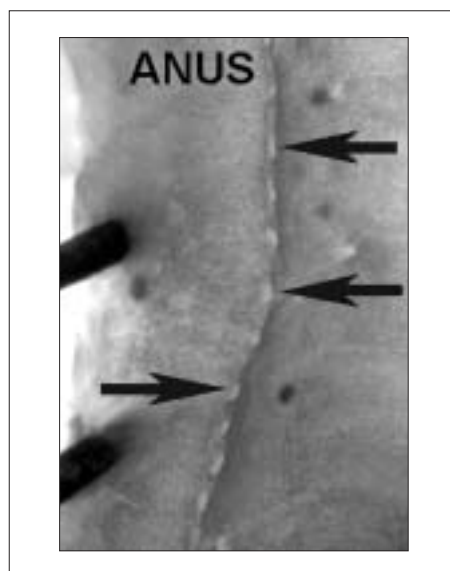


Figure 5. The perianal coelomoducts of *P. californicus*. Arrow points identify the exit openings of these ducts encircling the anus (ANUS), which may number in the hundreds in large representatives of this species.

medium. One potential metanephridial system that might facilitate osmoregulation of the coelomic fluid in *P. californicus* are the ciliated transrectal duct organs connecting the coelom to the outside medium (see Goodrich 1946; Ruppert and Barnes 1994; Ruppert and Smith 1988; Shinn 1985; Shinn et al. 1990). These ciliated coelomoducts can number in the hundreds in large specimens of *P. californicus* (Shinn et al. 1990), and connect the coelomic spaces near the insertion of the rectal suspensor muscles at the posterior wall of the rectum to the outside at the base of the anal fold. These ducts are located perianally and nominally function in removal of unwanted material from the coelom of *P. californicus* during seasonal visceral atrophy (Dybas and Fankboner 1986; Fankboner and Cameron 1985; Shinn 1985), and are believed to regulate the influx/efflux of the coelom's sea water (Shin et al. 1990). However promising, it remains to be established whether the ciliated transrectal duct organs could also function as osmoregulation organs in *P. californicus*.

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