Invertebrate Biology

Invertebrate Biology ▮▮(▮▮): 1–9.

DOI: 10.1111/j.1744-7410.2009.00182.x

A non-lethal method for estimation of gonad and pyloric caecum indices in sea stars

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Abstract. Gonad and pyloric caecum indices are widely used indicators of reproductive effort and nutritional condition in asteroids. Current methods of quantification generally require sacrificing multiple animals and the resulting reduction in local sea star density could have an unintended impact on benthic communities. Using the intertidal sea star *Pisaster ochraceus,* we developed and tested a method for estimating organ indices through the non-lethal sampling of single arms. Indices estimated via dissections of single arms accurately predicted the values obtained by sacrificing whole animals. In laboratory and field trials, we compared two methods of sampling single arms: (1) arm removal, and (2) organ extraction through an incision (without arm removal). Two years after these treatments, organs had regenerated in the affected arms of most sea stars, but were small relative to those in unmanipulated arms. The extent of organ recovery did not differ between the two treatments, but sea stars in the arm removal group were relocated in the field more frequently than those in the arm incision group. Our results suggest that sampling via the removal of single arms can be an effective, non-lethal method for estimating gonad and pyloric caecum indices in asteroids.

Additional key words: Asteroidea, reproduction, *Pisaster ochraceus*, regeneration

Numerous studies have investigated annual cycles of reproduction in a diverse group of asteroid species from around the world (e.g., Farmanfarmaian et al. 1958; Pearse 1965; Scheibling 1981; Franz 1986; Kettle & Lucas 1987; Guzmán & Guevara 2002). Additional research has examined intraspecific variation in the reproductive biology of sea star populations sampled in different habitats or years (Crump 1971; McClintock et al. 1988; Barker & Xu 1991; Sanford & Menge 2007). In these and many other studies, gonad indices have been used as a convenient measure of asteroid reproduction. Most sea stars have a pair of gonads and a pair of pyloric caeca (digestive organs) located in each arm. Resources stored in the pyloric caeca appear to play an important role in the seasonal production of gonads in some asteroid species (for a review, see Lawrence & Lane 1982). To quantify temporal and spatial variation in the size of the pyloric caeca and gonads, researchers have typically dissected the whole sea star to remove these organs from each arm. The gonad index and pyloric caecum index are then calculated by dividing the organ's total volume (or wet weight) by the wet weight of the animal (to remove the effect of body size on the estimates of relative organ size; Gonor 1972). The specific gravity of these organs and seawater are equivalent and thus indices determined by either organ volume or wet weight are comparable (Mauzey 1966).

Although the gonad index is a simple and effective means of quantifying reproductive effort, this method requires sacrificing the animal. Studies of annual reproductive cycles often require frequent collection of multiple individuals (generally 10 to 20 sea stars collected every 1–2 months) and therefore there is a risk of depleting local populations. Such impacts are of particular concern because many asteroids occur at relatively low densities, yet are top predators that play an important role in structuring intertidal and subtidal communities (for a review, see Menge 1982). Concern over depletion of local sea star populations has caused some researchers to limit sample sizes or restrict sampling to sites with large populations (e.g., Menge 1975; Scheibling 1981; Morgan & Cowles 1996), but these actions may limit statistical power and/or the scope of inferences that can be drawn.

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Asteroids have a remarkable capacity for regeneration and many species can readily replace arms lost to natural causes such as predation (Lawrence 1992; Lawrence & Larrain 1994). However, we are aware of only three studies that have calculated organ indices based on removal of a single arm (Pearson & Endean 1969; Pearse et al. 1986; Morgan & Cowles 1996), and these studies did not test whether data from one arm were representative of indices determined by whole-animal dissections. In this study, we used the ochre sea star *Pisaster ochraceus* BRANDT 1835 to examine whether organ indices can be quantified non-lethally and accurately through the sampling of single arms. *Pisaster ochraceus* is a keystone predator in rocky intertidal communities along the Pacific Coast of North America (Paine 1974; Menge et al. 1994). It is a broadcast spawning species with a long lifespan (estimated at >20 years) and a population density that varies considerably among sites (Menge 1975; Menge et al. 2004). The reproductive biology of *P. ochraceus* has been the subject of numerous studies that have used gonad indices (e.g., Feder 1956; Farmanfarmaian et al. 1958; Mauzey 1966; Pearse et al. 1986; Sanford & Menge 2007). Our study had two goals: (1) to determine whether organs removed from a single arm could be used to accurately estimate organ indices for the whole sea star, and (2) to evaluate two methods of non-lethal sampling to determine which was associated with the greatest survival and the fastest rate of organ regeneration.

Methods

Estimation of total organ volume

As part of a previous study of the reproductive ecology of *Pisaster ochraceus,* Sanford & Menge (2007) quantified organ indices at two wave-exposed, rocky intertidal sites on the central coast of Oregon, USA: Strawberry Hill (44°15′N, 124°07′W) and the Newport South Jetty (44°36′N, 124°04′W). Sea stars ($n = 12$ per site) were collected every other month between August 1995 and August 1997, and in five additional months during 1998–1999 ($n = 17$ sampling months, 419 sea stars total). Sampling was restricted to sea stars with a wet weight of 170–260 g, the most common size class of *P. ochraceus* at many sites in Oregon and California (Menge et al. 1994, 2004).

Following collection, sea stars were held in seawater tables for 24 h and then dissected to remove and quantify the volume of the gonads and pyloric caeca (for a full description of methods, see Sanford & Menge 2007). We tested whether the volume of organs removed from a single arm provided an accurate estimate of the total volume of organs in all five arms. Sea stars often have arms that vary somewhat in length and so we haphazardly chose an arm of intermediate length to be the representative single arm. A radial cut was made along the aboral surface of this arm using scissors, and the gonads and pyloric caeca were removed and blotted on paper towels for 15 min to remove excess water. The volumes of the gonads and pyloric caeca were then determined by their displacement of known volumes of water in a graduated cylinder (Mauzey 1966). Using the same methods, the other four arms were dissected to determine the total volume of the gonads and pyloric caeca from the entire sea star. For each sea star, the volume of gonads and the volume of pyloric caeca from the single arm were multiplied by five to obtain estimates of the total volumes. These estimates were then compared versus the actual total volumes using least squares linear regressions (JMP version 8.0, SAS Institute, Cary, NC, USA). Data from both field sites showed equivalent patterns and were pooled for analysis.

Drained wet weight and organ indices

Organ indices are often calculated by dividing the organ volume or weight by the drained (i.e., eviscerated) wet weight of the sea star (Lawrence & Lane 1982). This quantity can be represented as:

$$\begin{aligned}&\text{Drained wet weight}\\&\quad = \text{total wet weight} - \text{weight of gonads}\\&\quad - \text{weight of pyloric caeca}\\&\quad - \text{weight of coelomic fluid}\end{aligned} \tag{1}$$

Total wet weight is defined as the whole animal weight after blotting with paper towels, but before removal of any organs. We found that the total weight of the gonads and pyloric caeca can be estimated accurately by dissecting a single arm and multiplying these organ weights by five (see "Results"). Thus, the remaining unknown quantity in this equation was the weight of the coelomic (perivisceral) fluid. We tested whether this value could be estimated from the weight of coelomic fluid that drained from the body cavity during the non-lethal removal of one arm. During March 2007, we dissected individuals of *P. ochraceus* (wet weight = 150–300 g, $n = 48$) collected from four wave-exposed rocky intertidal sites in northern California: Pinnacle Gulch (38°18′N, 123°01′W), Bodega Marine Reserve (38°19′N, 123°04′W), MacKerricher State Park (39°28′N, 123°48′W), and Kibesillah Hill (39°35′N, 123°47′W). In the field, sea stars were placed in a shallow plastic container and one arm was removed. The sea star was tipped to drain as much coelomic fluid from the body as possible, and all fluid was transferred into a tared beaker and weighed on a portable electronic balance. We then completed the dissection of the whole animal, as described previously, and collected and weighed any remaining coelomic fluid. We used a least-squares linear regression to determine the relationship between the weight of fluid drained during arm removal and the total weight of coelomic fluid.

The resulting regression equation was used to estimate drained wet weights of each sea star, and estimated gonad indices from single arm dissections were calculated as

$$\text{Estimated gonad index} = \left[\frac{(\text{weight of gonads from one arm} \times 5)}{\text{estimated drained wet weight}}\right] \times 100 \quad (2)$$

Estimated pyloric caecum indices were calculated in an analogous manner. We then used linear regressions to compare these estimated values to organ indices quantified by dissection of the whole animal ($n = 48$ individuals).

Evaluation of methods: laboratory test

In late January 2007, we collected 30 individuals of *P. ochraceus* (wet weight = 150–260 g) from the Pinnacle Gulch site. Sea stars were returned to Bodega Marine Laboratory and each was given a temporary number. Numbers were drawn randomly to assign individuals to three treatments: "arm removal," "arm incision," and an unmanipulated control ($n = 10$ sea stars per treatment). In the arm removal treatment, scissors were used to remove the arm near to its attachment to the central disk. A scalpel was used to remove the gonads and the pyloric caeca from the amputated arm, as well as remnants of these organs attached just inside the central disk. In the arm incision treatment, the organs were extracted, but the arm was left attached to the sea star (Pearse et al. 1986). We hypothesized that the arm incision method would allow sea stars to regenerate organs more quickly than in the arm removal treatment because energy would not have to be expended on regeneration of the entire arm. For sea stars in this group, an intermediate length arm was selected and scissors were used to make a radial cut down the length of the aboral surface of the arm. Two short cuts were also made near where the arm was attached to the central disk, leading away at right angles from the radial incision. This allowed the walls of the arm to be folded back slightly while the gonads and pyloric caeca were removed using a scalpel. Following these treatments, we placed the sea stars in three 100-L aquarium tanks that were continuously supplied with flow-through seawater. We observed these individuals daily for the first 14 d to record their status and the time required to repair wounds. We continued to maintain sea stars in the laboratory for a 2-year period, occasionally adding mussels (*Mytilus californianus* CONRAD 1837) to each tank for food.

After 2 years, we evaluated the degree to which the affected arms of sea stars in the treatment groups (arm removal, arm incision) had regenerated organs of normal size. At this time, incisions were fully healed over, but were still discernable. Removed arms had partially regenerated and we recorded the radius of each arm (i.e., the distance from the center of the aboral disk to the tip of the arm). Using the methods described previously, all sea stars were dissected in March 2009 (the season when gonads are developing in this species; Sanford & Menge 2007; M.E. Wood, unpubl. data). Food provided during this study was not standardized among the three groups, and therefore we were unable to compare gonad and pyloric caecum indices among treatments and controls. Rather, we restricted our analyses to comparisons among arms within individual sea stars. For each individual, we calculated a gonad recovery index, defined as the ratio of gonad wet weight in the one affected arm to the mean gonad wet weight in the unmanipulated arms (i.e., total gonad wet weight/4 arms). Sea stars in the control group were dissected in the same manner except that, before dissecting each sea star, we haphazardly selected one focal arm to compare to the other four arms (because these individuals lacked affected arms). Analogous calculations were made to evaluate the weight of the pyloric caeca in affected versus unmanipulated arms in each of the three groups. Gonad and pyloric caeca recovery indices were then compared among the three groups (arm removal, arm incision, control) using two separate one-way analyses of variance (ANOVAs).

Evaluation of methods: field test

We also examined whether sampling of organ indices from a single arm led to increased loss of sea stars under field conditions. In nature, sea stars are challenged by a variety of conditions (e.g., exposure to waves, predators, desiccation at low tide, etc.) that might increase the mortality of sea stars following arm damage or loss. To test these impacts, we collected 45 individuals of *P. ochraceus* (wet weight = 150–300 g) from the Pinnacle Gulch site during a low tide in March 2009. Sea stars were randomly assigned to the same three groups (arm removal, arm incision, and unmanipulated control; $n = 15$ per group) and were treated in the field using procedures identical to those described for the laboratory trial. We then identified three large mid-intertidal boulders that were surrounded by sand (a feature that tends to slow large-scale movements of sea stars; but see Fager 1971) and removed all individuals of *P. ochraceus* that were present. Five sea stars from each of the three experimental groups were selected randomly and were placed at the lower edge of the mussel bed on each boulder. Individuals were splashed with seawater from a bucket until they had firmly reattached to the rock surface. We then returned to these boulders during low tide after 1, 2, 4, 6, 8, and 10 d to record whether sea stars in each group were present or absent. Variation among groups in the proportion of sea stars present on day 10 was analyzed using a one-way ANOVA.

Results

Estimation of total organ volume

Our data set of *Pisaster ochraceus* spanned both reproductive and non-reproductive seasons over a 4-year period and thus included a broad range of gonad and pyloric caecum volumes. The volume of gonads removed from a single arm accurately predicted the total volume of gonads from whole-animal dissections (Fig. 1A). Similarly, pyloric caecum volume within a single arm was a good predictor of the total volume of this organ (Fig. 1B).

Drained wet weight and organ indices

Based on field dissections ($n = 48$ sea stars), the weight of coelomic fluid collected during removal of one arm provided a reasonable estimate of the total weight of coelomic fluid within the entire sea star ($p < 0.0001$, $R^2 = 0.75$; total fluid weight = [$m \times$ weight of fluid captured during arm removal]+b; parameter estimates [SE]: $m = 1.074$ [0.093]; $b = 10.46$ [1.79]). By using this regression equation to estimate the total weight of coelomic fluid, and by weighing organs removed from single arms, we estimated gonad and pyloric caecum indices (Equation 2). The gonad index estimated by single arm dissection accurately reflected the true gonad index measured by whole-animal dissection (Fig. 2A). Similarly, the estimated pyloric caecum index was highly correlated with the actual pyloric caecum index (Fig. 2B).

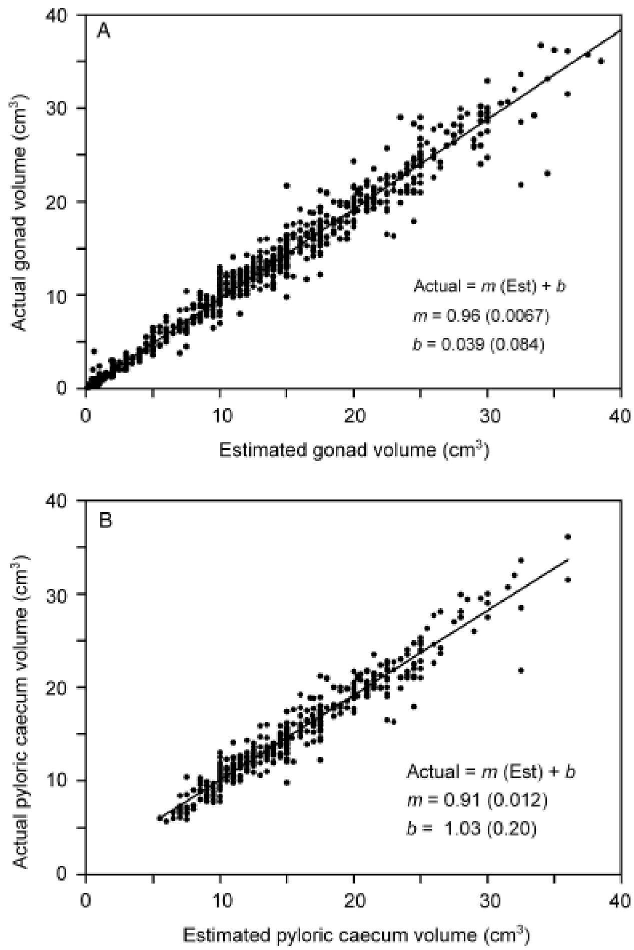

Fig. 1. Organ volumes estimated from dissection of a single arm versus dissection of the whole sea star, for (**A**) gonads and (**B**) pyloric caeca. Sea stars ($n = 419$) were collected from the central Oregon coast. Gonad and pyloric caecum volumes measured from a single arm were multiplied by five to estimate total volumes. Linear regressions were used to determine the relationships between actual and estimated (est) volumes, where m is the slope and b is the y-intercept. Numbers in parentheses are standard errors of the parameter estimates. Estimated organ volumes accurately predicted the actual volumes of gonads ($p < 0.0001$, $R^2 = 0.98$) and pyloric caeca ($p < 0.0001$, $R^2 = 0.93$) determined by dissecting the whole sea star.

Evaluation of methods: laboratory test

In the arm removal treatment, the body wall surrounding the amputated arm closed over the wound rapidly and completely sealed off this opening to the central disk within 1–12 h. In contrast, damage healed slowly for sea stars in the arm incision group. During the first few days following this treatment, the walls of the affected arm were often folded open, exposing the coelomic spaces inside the arm and the

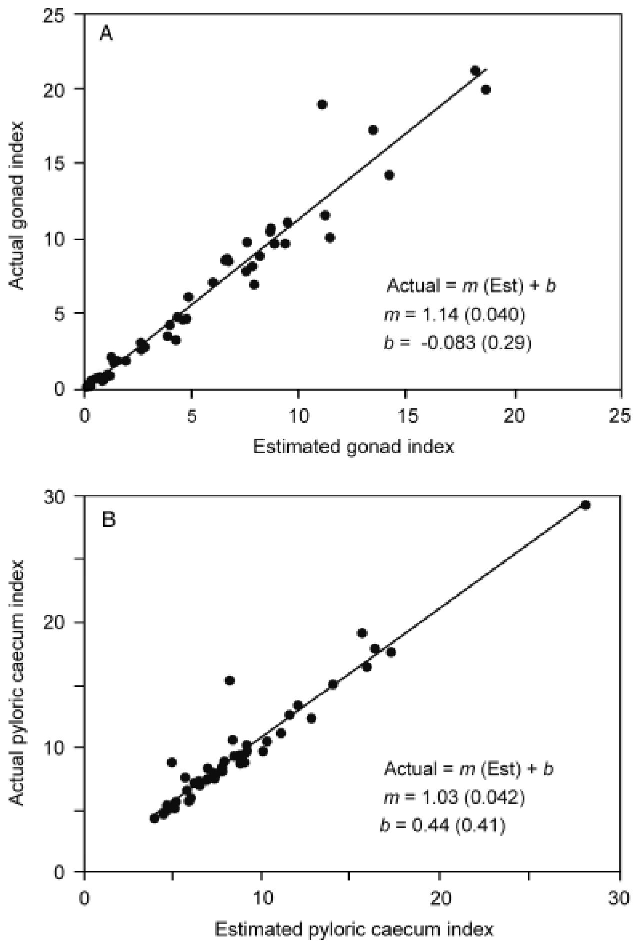

Fig. 2. A comparison of estimated versus actual organ indices ($n = 48$) for individuals collected from northern California. The gonad index (**A**) and pyloric caecum index (**B**) were estimated from dissection of a single arm. (See text for methods.) Actual indices were determined by dissection of the whole sea star and represent the total wet weight of the organ divided by the drained wet weight of the sea star (×100). Linear regressions were used to determine the relationships between actual and estimated (est) indices, where m is the slope and b is the y-intercept. Numbers in parentheses are standard errors of the parameter estimates. Estimated organ indices were strongly correlated with the actual values, for both the gonad ($p < 0.0001$, $R^2 = 0.95$) and pyloric caecum index ($p < 0.0001$, $R^2 = 0.93$).

central disk. In some animals, a small portion of the stomach extended out from this wound. After 6–7 d, the two sides of the incision were held together such that the coleomic spaces were generally sealed off. No sea stars autotomized arms or died during this laboratory trial (but see "Discussion").

After 2 years in the laboratory, individuals in the arm removal group had partially regenerated arms with radii that were 62–72% of the length of unmanipulated arms. As a measure of recovery within individuals, we compared the ratio of the weight of regenerated organs in the affected arm to the mean weight of organs in the four unmanipulated arms. Negative impacts on gonad weight were strong in the arm removal and arm incision groups and, as expected, were absent in the control group (Fig. 3; ANOVA, $F_{2,26} = 411.29$, $p < 0.0001$). The magnitude of these negative effects did not differ between the arm removal and arm incision treatments (Tukey–Kramer, $p > 0.05$). In both treatments, the focal arm (i.e., whether regenerating or with a healed incision) had very small gonads that were only ~4% of the mean wet weight of those organs averaged across the individual's other four arms.

The arm removal and arm incision treatments also had a strong negative impact on the wet weight of pyloric caeca within affected arms, whereas the pyloric caeca differed little among the arms of control sea stars (Fig. 3; ANOVA, $F_{2,27} = 294.03$, $p < 0.0001$). After 2 years of recovery, pyloric caecum wet weights in affected arms were only ~11–12% of those in unmanipulated arms, and these negative

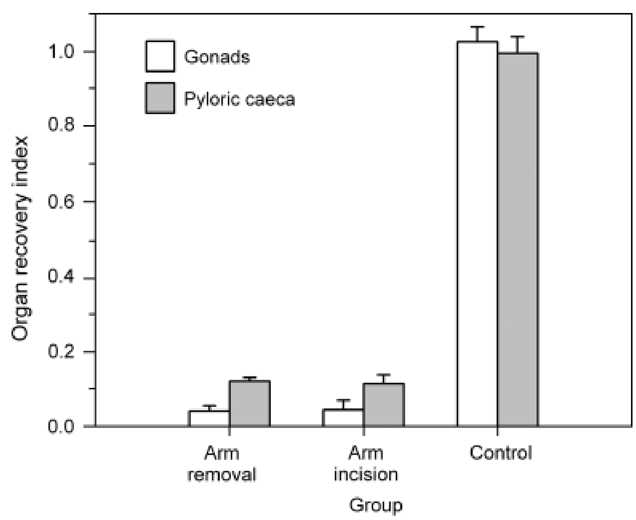

Fig. 3. Regeneration of gonads and pyloric caeca after these organs were removed using two different methods. Bars show the mean recovery (+SE) of the gonads and pyloric caeca 2 years after these organs were removed from a single arm by (**A**) removal of the whole arm, or (**B**) through an arm incision that left the arm attached. Sea stars in the control group received no treatment; $n = 10$ sea stars per group. The organ recovery index is the ratio of the organ wet weight in the focal (i.e., affected) arm divided by the mean wet weight of that organ in the four unmanipulated arms of the same sea star. For control sea stars (that lacked affected arms), we haphazardly selected one arm to be the focal arm for these calculations. (See text for statistical results.)

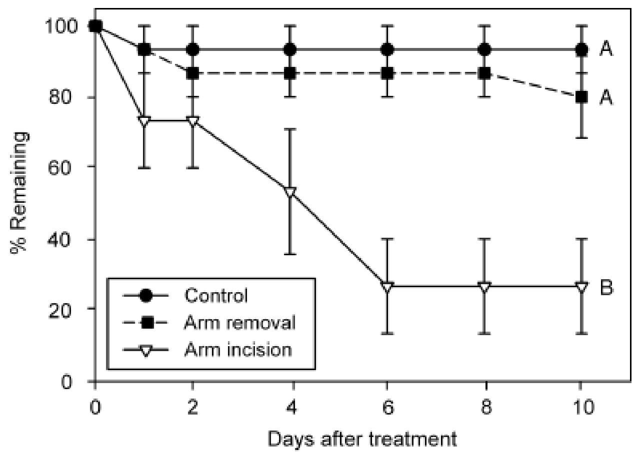

Fig. 4. Percent of individuals remaining in the field following sampling of single arms. Sea stars were subjected to either: arm removal, arm incision, or no removal of organs (control). Following these treatments, five individuals per group were randomly assigned to three isolated, mid-intertidal boulders at Pinnacle Gulch, CA, and were monitored for 10 d. For each group, data are mean percents (±SE) of sea stars present on each survey date ($n = 3$ boulders). Shared letters to the right of the final data points indicate groups for which the mean percent remaining did not differ on day 10 (Tukey–Kramer, $p > 0.05$).

effects did not differ between the arm removal and arm incision treatments (Tukey–Kramer, $p > 0.05$).

Evaluation of methods: field test

In the field trials, we relocated fewer sea stars from the arm incision group than from either the arm removal or control groups (Fig. 4; ANOVA, $F_{2,6} = 10.50$, $p = 0.011$). After 10 d, only 26.7% of individuals with arm incisions remained, whereas the percentages of sea stars remaining were much higher and did not differ between the arm removal and control groups (80.0% and 93.3%, respectively; Tukey–Kramer, $p > 0.05$).

Discussion

Estimation of organ indices

Our results suggest that sampling of single arms can be an effective, non-lethal method for estimating organ indices in sea stars. The total volumes of both gonads and pyloric caeca in *Pisaster ochraceus* were predicted accurately by dissection of one arm, indicating that variation in organ size among arms is small. Organ indices are often calculated using the ratio of organ volume (or weight) to the drained wet weight of the sea star, the approach that we followed in this study. Other researchers have instead divided organ volume by the total wet weight of the sea star, measured before dissection (for a review, see Lawrence & Lane 1982). However, organ indices calculated in this manner tend to reduce variation among individuals because organ weights are included in both the numerator and the denominator. In addition, total wet weight includes the weight of coelomic fluid, a contribution that ranges 8–34% of the total wet weight of 150–300 g individuals of *P. ochraceus* (M.E. Wood, unpubl. data; S. Pincebourde, unpubl. data). This percentage can vary strongly among individuals, and can also change considerably in this and other asteroids over time scales ranging from days to months (Pearse 1967; Franz 1986; Pincebourde et al., in press). Thus, we regard drained wet weight as a more consistent and reliable measure of sea star size than total wet weight. We determined that this value can be estimated without dissecting the whole animal using the equation:

$$\begin{aligned}\text{Drained wet weight} &= \text{total wet weight} \\ &- (\text{weight of gonads from one arm} \times 5) \\ &- (\text{weight of pyloric caeca from one arm} \times 5) \\ &- [(1.074 \times \text{weight of fluid captured during arm removal}) + 10.46]\end{aligned} \tag{3}$$

Given an estimate of drained wet weight, the gonad index can then be estimated following Equation (2). Similarly, the pyloric caecum index can be estimated as:

$$\text{Estimated pyloric caecum index} = \left[\frac{(\text{weight of pyloric caeca from one arm} \times 5)}{\text{estimated drained wet weight}}\right] \times 100 \tag{4}$$

Evaluation of methods of arm removal

The results from laboratory and field trials suggest that whole arm removal is the most effective method for sampling organs from a single arm. The removal of a whole arm is similar to arm loss via autotomy, a response induced in some sea stars by predator-related damage or other disturbances (Lawrence 1992; Lawrence & Larrain 1994). Sea stars rapidly closed off wounds from whole arm removal. In contrast, sea stars in the arm incision group had open wounds that exposed coelomic spaces for a week or longer. In the laboratory trial described in this article, none of the sea stars in the arm incision treatment autotomized the damaged arm. However, when we repeated this treatment in a later year, six of ten sea stars autotomized the damaged arm and later died. Although the reason for this variation between trials in response to arm incisions is unknown, the potential for autotomy and mortality following this treatment raises serious concerns about this method. Similarly, in the field trials, we relocated a smaller percentage of sea stars in the arm incision group than in either the arm removal or control groups, suggesting that animals in this group may have experienced greater mortality. Our findings are consistent with a previous laboratory study of *P. ochraceus* (Pearse et al. 1986), indicating that removal of organs through an incision led to mortality, whereas animals healed well following whole arm removal.

After 2 years in the laboratory, gonads and pyloric caeca were present in almost all arms affected by the arm removal and incision treatments, confirming that individuals of *P. ochraceus* have the capacity to regenerate these organs. However, recovery of organ size was slow in both of these treatments. Sea stars held in our laboratory study were provided food on an occasional basis (rather than *ad libitum*), and the resulting growth was comparable to that reported for marked individuals observed at field sites in Monterey Bay, CA (E. Sanford, unpubl. data; Feder 1970). Thus, the rates of regeneration that we observed in the laboratory may be similar to those that would occur in nature. These results are consistent with previous work in asteroids indicating that the pyloric caeca regenerate slowly, and that the costs of regeneration and reduced energy stores may reduce gonad production (Harrold & Pearse 1980; Lawrence et al. 1986; Lawrence & Larrain 1994). Given the long lifespan of this sea star (estimated at >20 years; Menge 1975), it is likely that the pyloric caeca and gonads in the sampled arms would recover greater size and function over time.

We hypothesized that organ removal through an incision would allow sea stars to regenerate these organs more quickly than in the arm removal group, because most of the arm was left intact. However, this was not the case; the ratio of regenerated to unmanipulated organ weights did not differ between the arm removal and arm incision treatments. Given these equivalent rates of recovery, and the fact that arm incisions healed slowly and were associated with a greater loss of individuals in the field, we recommend removal of the entire arm as the preferred method for single arm sampling.

Conclusions

Our results demonstrate that non-lethal sampling of single arms can provide an accurate estimate of organ indices in *Pisaster ochraceus* while minimizing the ecological impacts that might be caused by the local depletion of this keystone predator. In addition, our method has the added benefit of reducing researcher labor, as removal of a single arm is considerably faster than the time-consuming dissection of the entire sea star. Presumably, the non-lethal approach developed here can be applied readily to other asteroid species. In this case, we suggest that researchers conduct whole-animal dissections on a small number of individuals to establish species-specific equations and confirm the reliability of this technique. In particular, other species may vary in the relationship between the weight of coelomic fluid released from removal of one arm versus the total weight of coelomic fluid present.

In recent years, there has been growing interest in quantifying variation in reproductive output among marine populations, to inform spatial management and improve understanding of source–sink dynamics in marine species (e.g., Rogers-Bennett et al. 1995; Leslie et al. 2005; Sanford & Menge 2007). Sea stars often play an important ecological role in marine benthic communities (Menge 1982), and thus asteroids are a logical choice to include among those taxa that are assessed for variation in reproductive output. Given that arm removal imposes energetic costs on asteroids (Lawrence et al. 1986; Lawrence & Larrain 1994), there may be some risks of obtaining biased data if individuals that were manipulated previously are later re-sampled during subsequent surveys of a population. However, avoiding individuals with regenerating arms should ensure that individuals are not sampled during the early phases of recovery (when energetic costs are likely to be the greatest). We hope that the application of the sampling method described here will facilitate continued research on the reproductive ecology of asteroids while minimizing the potential ecological impacts associated with repeated removal of sea stars from local populations.

Acknowledgments. We thank K. Sato and M. Church for field and laboratory assistance, and B. Menge, S. Pincebourde, and J. Sones for helpful suggestions. This article was improved by constructive comments from J. Pearse and an anonymous reviewer. This research was funded by a National Science Foundation graduate research fellowship and NSF grant OCE-06-22924 to E.S., and an Environmental Protection Agency

STAR graduate fellowship to M.W. This is contribution number 2457 of the Bodega Marine Laboratory, University of California, Davis, USA.

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