Mar Biol (2011) 158:47–58
DOI 10.1007/s00227-010-1541-2

ORIGINAL PAPER

Problems of the gonad index and what can be done: analysis of the purple sea urchin *Strongylocentrotus purpuratus*

T. A. Ebert · J. C. Hernandez · M. P. Russell

Received: 2 April 2010 / Accepted: 30 August 2010 / Published online: 14 September 2010

Abstract The gonad index, GI, is widely used as a measure of changes in reproductive state. There are, however, problems with its use because it is based on the implicit assumption of an isometric relationship between gonad size and some measure of total size. If, for example, gonad weight and total weight are used, the exponent for an allometric relationship usually is ignored and hence assumed to be 1.0. It is further assumed that this exponent is fixed for all states of the reproductive cycle and that gonads begin to develop at size = 0. Data for the purple sea urchin *Strongylocentrotus purpuratus* at Gregory Point, Oregon, USA, gathered over a period of 31 months showed that these assumptions cannot be supported. The relationship is better modeled with a function that (1) takes into account size of initial gonad production and (2) allows allometric exponents that vary with site or season. Thus, a better approach is to use a wide range of sizes to estimate size when gonads begin to develop and then, with this correction, ANCOVA to test for differences of gonad size among samples. Gonad changes at Gregory Point were estimated using fixed sizes of 5 cm diameter and 60 g total weight. Publishing means for *X* and *Y*, the standard error of the estimate, R^2, and slope for each regression are shown to be sufficient to compare our results with results across studies.

Communicated by M. Byrne.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-010-1541-2) contains supplementary material, which is available to authorized users.

T. A. Ebert (✉)
Department of Zoology, Oregon State University,
Corvallis, OR 97331-2914, USA
e-mail: ebertt@science.oregonstate.edu

J. C. Hernandez · M. P. Russell
Department of Biology, Villanova University,
Villanova, PA 19085-1699, USA

Present Address:
J. C. Hernandez
Departamento de Biología Animal (Ciencias Marinas),
Universidad de La Laguna, Tenerife, Islas Canarias

Introduction

Describing morphological changes in body parts with ratios is pervasive in biology and has been used for decades. Criticism of ratios or body indices also is of long standing (e.g., Atchley et al. 1976; deVlaming et al. 1982) and consensus of critics is that ANCOVA is a superior approach to analysis (e.g., Packard and Boardman 1987, 1999; Raubenheimer and Simpson 1992). Comparison of analyses using ratios and ANCOVA has shown that errors due to the statistical analysis of ratio-based indices may be more serious than generally appreciated (Beaupre and Dunham 1995). Nevertheless, ratios are intuitive and easy to calculate whereas "…use and interpretation of ANCOVA requires a greater statistical sophistication …" (Liermann et al. 2004). With the nearly universal availability of statistical software, however, ANCOVA is readily available and not difficult to use with the sorts of data sets associated with changes in sizes of body parts. Work we present here focuses on use of a gonad index in sea urchins but the problem is very general because dissections of many organisms are done for various reasons and indices continue to be the summary of choice.

The gonad index, gonosomatic index, or gonadosomatic index, GI, has taken a variety of forms for marine invertebrates (Table 1) including wet and dry weights (e.g.,

Table 1 Examples of ways a gonad index, GI, has been calculated for different marine invertebrates and usually expressed as percent; this list is not exhaustive

Invertebrate group	References
Echinodermata	
Echinoidea	
Gonad wt wet/total wt wet	Lawrence et al. (1965), Giese (1966), Pearse (1968), Vernon et al. (1993), Vadas et al. (2000), McCarthy and Young (2002), Bigatti et al. (2004), Lester et al. (2007)
Gonad vol/total wt wet	Bennett and Giese (1955), Giese et al. (1959)
Gonad wt dry/total wt dry	Excluding body fluid and gut contents. Gonor (1972)
	Including gut contents. Laegdsgaard et al. (1991), Raymond and Scheibling (1987), Guillou and Lumingas (1999)
Gonad vol/test vol	Moore et al. (1963), McPherson (1965), Lewis and Storey (1984)
Gonad vol/test diameter	Lewis (1958)
Gonad vol/test diameter^3	Lewis (1966)
Gonad vol/body wt dry without gut contents	Lessios (1981)
4 gonads wt dry/body wt dry	Body is without gonads and gut, Lozano et al. (1995)
Gonad wt wet/drained body wt wet	Kramer and Nordin (1975), Bernard (1977)
Gonad wt wet/eviscerated test wt wet	Kelly et al. (2001), MacCord and Ventura (2004)
Gonad wt dry/test diameter^3	Guettaf et al. (2000)
Gonad wt dry/test diameter^2 height	Ebert (1968)
Gonad wt dry/eviscerated test wt dry	Guillou and Lumingas (1998)
Ophiuroidea	
Gonad wt dry/eviscerated body wt dry	"Body" excludes arms, gut, and gonads; Bourgoin and Guillou (1990)
Gonad wt dry/total weight dry	Lefebvre et al. (1999)
Asteroidea	
Gonad wt wet/total wt wet	Lawrence (1973), Boivin et al. (1986), Chen and Chen (1992), Byrne et al. (1997)
Gonad wt dry/eviscerated body wt dry	"Eviscerated" = no gonads or pyloric cacea; Barker and Xu (1991), Byrne (1992)
Gonad wt dry/body wt dry (without gonads)	Nojima (1979)
Gonad vol/eviscerated wt wet	Without gonad and pyloric caeca; Town (1980), Nichols and Barker (1984)
Holothuroidea	
Gonad wt wet/body wall wt wet	Chao et al. (1995)
Gonad vol/body wall wt wet	Body weight is without viscera or body fluid; Cameron and Fankboner (1986)
Gonad wt wet/gutted body wt wet	Gutted body is whole animal minus gut and gonad; Gutt et al. (1992)
Gonad wt wet/total body drained of fluid	Ong Che and Gomez (1985)
Arthropoda	
Crustacea	
Gonad wt dry/carapace width	Diez and Lovrich (2010)
Gonad wt wet $\times$ $10^5/L^3$ (L = carapace length)	Pérez-González et al. (2009)
Gonad wt wet/carapace length	Quackenbush and Herrnkind (1981)
Cnidaria	
Gonad wt dry/total wt dry	Chadwick-Furman et al. (2000)
Gonad vol/total wt wet	Ford (1964)
Mollusca	
Cephalopoda	
Nidamental gland or testis (mm)/mantle (mm)	Costa and Fernandes (1993)
Gonad wt wet/total body wt wet	Hatfield and Murray (1999)

Table 1 continued

Invertebrate group	References
Bivalvia	
Gonad wt wet/total tissue wt wet	Hines (1979)
Mantle wt dry/(body-mantle) wt dry	Nicastro et al. (2010)
Gastropoda	
Gonad wt wet/somatic tissue wt wet	Liu (1994)
Annelida	
Gonad energy/total body energy in joules	Olive et al. (1997)

Lawrence et al. 1965; Gonor 1972), volumes (e.g., Moore et al. 1963), linear measurements (e.g., Costa and Fernandes 1993) and units of energy (e.g., Olive et al. 1997). Mixtures of linear and weight measurements may or may not include exponents. For example, in studies of Crustacea, carapace width or length has been used without an exponent (Diez and Lovrich 2010) or with an assumed exponent of 3 (Pérez-González et al. 2009). With bivalves, the mantle may or may not be included in the denominator (Hines 1979; Nicastro et al. 2010).

For ophiuroids, body weight may mean total (Lefebvre et al. 1999) or excluding arms, gut, and gonads (Bourgoin and Guillou 1990). Similarly in asteroids, body weight may mean total (Boivin et al. 1986; Chen and Chen 1992; Byrne et al. 1997) or eviscerated with gonads removed (Nojima 1979) or both gonads and the pyloric cacea removed (Barker and Xu 1991; Byrne 1992). Measures could be either wet or dry. Gonad indices for holothurians generally use the body drained of fluid (e.g., Ong Che and Gomez 1985) but may also include removal of gut and gonad (e.g., Cameron and Fankboner 1986; Gutt et al. 1992).

In studies of sea urchins (echinoids), measurements have been of whole body weight and gonad volume (Bennett and Giese 1955), volume for both gonads and body (Moore et al. 1963), dry weights sometimes excluding gut contents (Gonor 1972) and sometimes including gut contents (Laegdsgaard et al. 1991). Sometimes only some of the five gonads have been used (Lozano et al. 1995; Byrne et al. 1998). When linear and weight measurements have been mixed, diameter has been cubed (e.g., Lewis 1966; Guettaf et al. 2000), sometimes not (Lewis 1958), and may include height as well as diameter (Ebert 1968). Finally, in some studies, body wet weight was used following evisceration or draining of body fluid (Kramer and Nordin 1975; Kato and Schroeter 1985; Guillou and Lumingas 1998; Kelly et al. 2001).

Over the years, analysis for sea urchins has settled on using wet weight of the gonad divided by the total wet weight multiplied by 100% (e.g., Hughes et al. 2006; Kirby et al. 2006; Lester et al. 2007; Lau et al. 2009), which is

$$\mathrm{GI} = \mathrm{gonad}/\mathrm{total} \times 100\%. \tag{1}$$

The general form of the gonad index (GI), however, is an allometric equation (power function) of gonad size (G) and some measure of total body size (S)

$$G = \alpha S^{\beta} \tag{2}$$

and, with the assumption that $\beta = 1.0$, α is what Byrne et al. (1998) have called the "gonad retrieval rate". The parameter α is the slope of a linear regression of gonad weight as a function of total weight with a 0-intercept. Rearrangement of Eq. 2 is

$$\alpha = G/S^{\beta} \tag{3}$$

and α is the gonad index, GI; the "gonad retrieval rate" is the same as Eq. 1. Equation 3 is the form of all of the examples in Table 1.

The first problem is that for Eq. 3 to be the same as Eq. 1, the exponent β must be equal to 1.0. If size, S, is a linear measurement the exponent β may be arbitrarily assigned the value 3 (e.g., Lewis 1966; Guettaf et al. 2000; Pérez-González et al. 2009) or may be used without an exponent, which assumes that β is 1.0 (Diez and Lovrich 2010). Damián et al. (2009) calculated β then fixed the value for an ANCOVA with a restricted set of size classes.

A second problem is that gonads begin to develop at a size that is not equal to zero and so Eq. 2 requires an additional parameter

$$G = \alpha(S - C)^{\beta} \tag{4}$$

where C is the size when gonads begin to develop. In general, this problem is not addressed (e.g., Diez and Lovrich 2010). Equation 4 has, however, been used to estimate C for several sea urchin species (Ebert and Russell 1994; Russell 1998; Ebert 1998, 2008, 2010b; Selden et al. 2009) but interest in the general problem of change in relative growth of gonads is of long standing (e.g., Robb 1929).

Additional problems with Eq. 1 center on the use of ratios when the independent variable appears as X but also is used in Y (cf. Atchley et al. 1976) as is the case where a body index is plotted as a function of total size to show a size-dependent relationship (e.g., Gonor 1972). "Part-whole correlation" (Christians 1999) where total weight includes gonad weight, and the total weight changes as gonad weight changes, is not a problem in sea urchin studies: total weight is independent of gonad weight because of the inverse relationship between body fluid and gonad weight. This inverse relationship, however, is a problem with the calculation of a gonad index, GI, using drained weight (Kramer and Nordin 1975; Bernard 1977).

There are various uses of the GI with the most basic being a simple description of a periodic, usually annual, reproductive cycle (e.g., Bennett and Giese 1955; Lawrence et al. 1965; Pearse 1968; Lewis and Storey 1984; Bigatti et al. 2004). Although not certain, the GI probably is suitable if this is the only goal of the study. When comparisons of the GI using additional statistical analyses are made across sites (e.g., Guillou and Lumingas 1999; Lester et al. 2007), sizes (e.g., Gonor 1972), or seasons (e.g., Laegdsgaard et al. 1991) failure to use an appropriate allometric model may influence conclusions (cf. Beaupre and Dunham 1995).

Our goal is to show problems associated with application of a body index, and the gonad index in particular. We also demonstrate the use of ANCOVA in analysis of a 31-month study of gonad changes in the US west coast purple sea urchin *Strongylocentrotus purpuratus* and finally show how analysis in this manner permits use of the results in this paper to test for differences in future studies. In this regard, it is a problem of selecting the minimum number of summary statistics that need to be published in order to do a statistical analysis without access to raw data although raw data always will be best.

Methods

Purple sea urchins (*Strongylocentrotus purpuratus*) were collected monthly during low tides at Gregory Point, Oregon (43° 20′ N; 124° 22′ W) from January 2007 through July 2009. Test diameter was measured with digital calipers, total wet weight determined, and gonads removed and weighed wet. A range of sizes was collected each month and during the entire study spanned 2.5–9.2 cm for test diameter and 8.3–298 g for total wet weight.

The first step in analysis was to estimate the parameter C for both diameter and total wet weight in Eq. 4, which was done by nonlinear regression (NONLIN in SYSTAT 2004). Samples were selected that had individuals sufficiently small that estimates of C were less than 1 cm or 1 g total wet weight and included some from Gregory Point, Boiler Bay, Yaquina Head and Sunset Bay in Oregon and Arena Cove and Yankee Point in California. The model used was

$$\ln G = A + \beta \ln(S - C) \quad (5)$$

where G is gonad wet weight (g), A is $\ln\alpha$, and S is either diameter (cm) or total wet weight (g). A logarithmic transformation was used to minimize the influence of increasing variation in gonad sizes of large individuals on the estimate of C.

Following the determination of C, the next step was to test whether a single exponent, β, was appropriate for all monthly samples, which was done by an ANCOVA using the general linear model (GLM) in SYSTAT. There are 31 monthly samples and all could have the same value of β or β could change as gonads increase and decrease in weight. The model can be thought of as making A and β in Eq. 5 linear functions of the sample number (month) that goes from January 2007 (sample #1) to July 2009 (sample #31). Sample number was treated as a descriptive factor in the analysis and not ordinal. Samples could have been called *a*, *b*, *c*, etc. Including "sample" in the analysis is

$$A = b_1 \text{sample} + c_1 \quad (6)$$

$$\beta = b_2 \text{sample} + c_2 \quad (7)$$

Substituting Eqs. 6 and 7 into Eq. 5 is

$$\ln G = b_1 \text{sample} + c_1 + (b_2 \text{sample} + c_2) \times \ln(S - C) \quad (8)$$

or

$$\ln G = b_1 \text{sample} + c_1 + c_2 \ln(S - C) + b_2 \text{sample} \times \ln(S - C). \quad (9)$$

The question is whether the interaction term, sample $\times$ $\ln(S - C)$, can be viewed as the same for all samples and this can be answered by using ANCOVA or by multiple regression. We used ANCOVA. If the interaction term of sample $\times$ $\ln(S - C)$ cannot be considered to be the same for all samples, each sample needs a separate regression.

Comparisons across sites were done by selecting a fixed size for diameter or total wet weight. A convenient way of doing this and obtaining 95% confidence limits for gonad weight is by nonlinear regression. In SYSTAT, as part of the estimation of A and β (Eq. 5), an additional line of code can be added as a function of parameters. We used a diameter of 5 cm and total wet weight of 60 g, which are values well within the range of purple sea urchin sizes along the Pacific coast of North America (Ebert 2010a). Using diameter as the covariate

$$\text{FUNPAR G\_5} = A + \beta * \text{LOG}(5 - C) \quad (10)$$

at a diameter of 5 cm or

$$\text{FUNPAR G\_60} = A + \beta * \text{LOG}(60 - C) \quad (11)$$

with total wet weight used as the covariate and 60 g as the fixed size. G_5 and G_60 are the ln gonad weights for these sizes. For comparison, analysis was also done using Eq. 4 without a logarithmic transformation because a logarithmic transformation weights small sizes and decreases the contribution to variation of large individuals.

An alternative to using FUNPAR in SYSTAT is to use the linear regressions of ln gonad weight as a function of ln(5 − *C*) for diameter or ln(60 − *C*) for total weight and then use the estimated slopes and intercepts to calculate ln gonad weight as well as the 95% confidence limits for the estimate at 5 cm or 60 g or other value of *X* (cf. Zar 1974 or any statistics test).

A final problem was how to present information so that it could be compared with other studies. The best approach always is to use the raw data, but much can be done with summary statistics. Our solution is to focus on the minimum number of summary statistics required so results presented here can be used by other investigators to test for differences in gonad development.

Results

Dissection data used to estimate *C* (Eq. 4) with size, *S*, as diameter measured in centimeters (Fig. 1) included individuals sufficiently small that a reasonable estimate of *C* could be obtained. A difficulty was with the dissection of gonads in very small individuals and the resolution of different balances that were available at laboratories. Estimates of *C* (Table 2) were 0.7 cm for diameter and 0.5 g for wet weight and used with Eqs. 4 and 5 in the following analyses.

An example of data for Gregory Point (Fig. 2) shows seasonal changes from January–December 2007 with

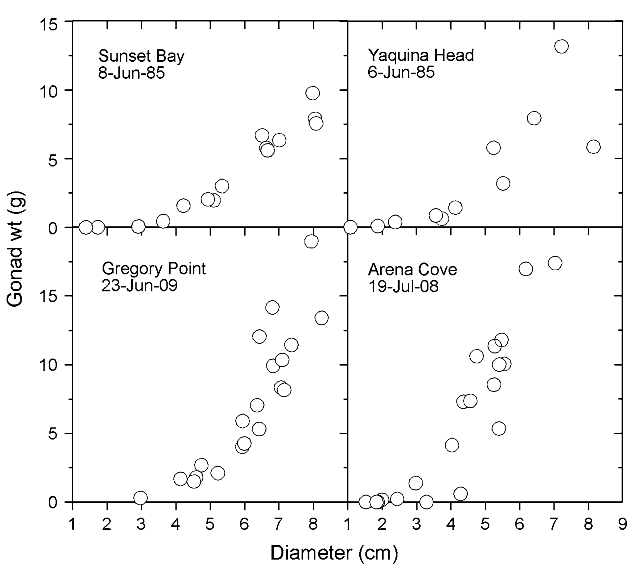

Fig. 1 Four of the samples used for *Strongylocentrotus purpuratus* to estimate *C*, the diameter when gonads begin to develop (Table 2)

Table 2 Estimates of *C*, the size when gonads begin to develop

Site	Date	*N*	R^2	*C* (cm)	SE
A. Diameter (cm)					
Sunset Bay, OR	8-Jun-85	14	0.97	0.858	0.397
Yaquina Head, OR	6-Jun-85	10	0.92	0.690	1.112
Yaquina Head, OR	17-May-87	10	0.97	0.411	1.053
Gregory Point, OR	16-May-07	20	0.95	0.519	1.088
Gregory Point, OR	23-Jun-09	20	0.93	0.747	1.337
Arena Cove, CA	16-Jan-07	20	0.89	0.858	1.017
Arena Cove, CA	5-May-07	20	0.84	0.627	1.815
Arena Cove, CA	15-Jul-07	20	0.92	0.894	0.435
Arena Cove, CA	18-Jul-08	20	0.76	0.879	0.937
Arena Cove, CA	30-Sep-08	20	0.83	0.869	0.652
Arena Cove, CA	17-Oct-08	20	0.8	0.597	0.408
Arena Cove, CA	25-Nov-08	20	0.89	0.544	0.414
			Mean =	0.708 cm	
Site	Date	*N*	R^2	*C* (g)	SE
B. Total wet weight (g)					
Boiler Bay, OR	12-Aug and 15-Oct-07	40	0.92	0.499	0.746
Yaquina Head, OR	17-May-87	10	0.97	0.875	2.535
Yankee Point, CA[a]	Dec-65	16	0.98	0.057	0.368
Arena Cove, CA	25-Nov-08	20	0.89	0.455	0.432
			Mean =	0.471 g	

[a] Based on data from Giese (1967)

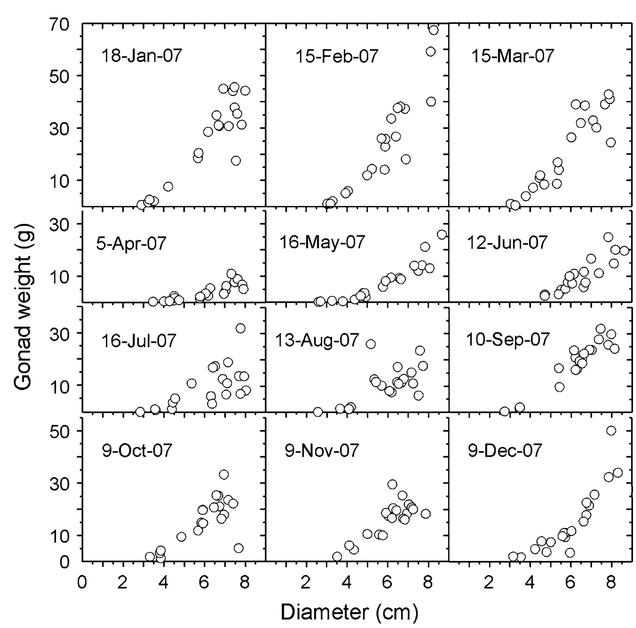

Fig. 2 Monthly dissections of *Strongylocentrotus purpuratus* at Gregory Point, Oregon, showing increasing spread of data points with increasing diameter and the presence of outliers particularly in August and October

Table 3 ANCOVA for monthly gonad dissections of *Strongylocentrotus purpuratus* at Gregory Point, Oregon from January 2007–August 2009; 20 urchins were dissected for each month

Source	SS	*df*	MS	*F*-ratio	*P*
A. Diameter measurements in centimeters					
ln (Dia − 0.7)	449.499918	1	449.4999	3281.373	<0.000001
Sample	20.384785	30	0.67949	4.9603	<0.000001
ln (Dia − 0.7) × Sample	11.826195	30	0.39421	2.8777	0.000001
Error	76.437806	558	0.13699		
Source	SS	*df*	MS	*F*-ratio	*P*
B. Total wet weight in grams					
ln (Total − 0.5)	454.91795	1	454.91795	3321.3185	<0.000001
Sample	21.164651	30	0.705488	5.150712	<0.000001
ln (Total − 0.5) × Sample	13.02493	30	0.434164	3.169798	<0.000001
Error	76.428748	558	0.136969		

highest values in February and lowest in April. Variation in gonad weights increased with diameter and included outliers such as a high gonad weight in August and a very low gonad weight in October. Outliers were included in all analyses. An ANCOVA for all 31 monthly samples (Table 3) for both independent variables ln(Dia − 0.7) and ln(Total − 0.5) showed that in both cases the interaction of Sample × Independent Variable (diameter or total wet weight) indicated that a common slope was not appropriate for further analysis ($P < 0.00001$). Consequently, individual regressions were used for each sample.

A first approach was to examine slopes for patterns. If just one or two slopes were very different then it still may have been reasonable to continue with a common slope. A pattern of slopes, however, was clear both for ln(Dia − 0.7) and ln(Total − 0.5) (Fig. 3) with high and low points that showed an annual cycle. The largest slope, β, occurred in June 2007, which was 2 months after the smallest gonads in April 2007 (Fig. 2). The smallest slope (Fig. 3) was in November 2007, 2–3 months before the largest gonads were detected. It is clear (Fig. 3) that use of diameter or total wet weight provide nearly identical patterns and neither provide exponents that consistently correspond with the expected values of 3.0 for diameter or 1.0 for total wet weight.

An approach to comparing sites or samples is to select some size, either diameter (5.0 cm) or total wet weight (60 g), and estimate the gonad wet weight for such a fixed size. This summary, which is virtually identical for diameter or total wet weight (Fig. 4) shows similar patterns of

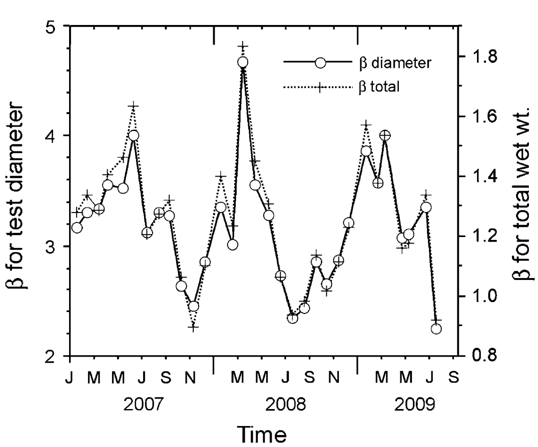

Fig. 3 Monthly changes of the allometric exponent β with analyses of diameter or total wet weight as covariates and with corrections, *C*, for when gonads begin to develop

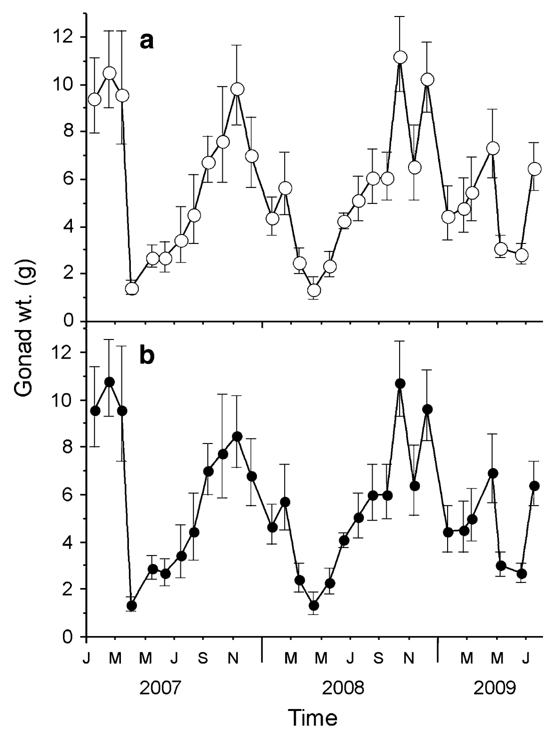

Fig. 4 Change in gonad weight for *Strongylocentrotus purpuratus* over 31 months at Gregory Point, Oregon; **a** diameter fixed at 5 cm; **b** total wet weight fixed at 60 g; *error bars* are 95% confidence limits

peaks and valleys across years but also yearly differences using either diameter or total wet weight with respect to times when gonad weight drops as well as the rate of this change. An interesting feature of the graph is the differences in the 95% confidence intervals, which are largest when gonads are large and smallest when gonads are small. This pattern is not surprising in examining the 2007 data (Fig. 2) where the spread of data points for large individuals is greatest when gonads are large.

The choice of using logarithmic transformation has consequences for how lines pass through the data points because small values are given greater weight in analysis. Differences between transformed and nontransformed data often are not great but in some cases fitted lines can be very different. Data for the first 4 months of the study (Fig. 5) show results for both transformed and untransformed data and for both diameter and total wet weight measurements of the covariate. In all cases using transformed data, a better fit for small individuals is provided and so initially in all cases the line for untransformed data lies above the line for transformed data. In some instances (5 April 2007), relative position remains for all sizes, diameter or total wet weight. For other samples, the lines for transformed and

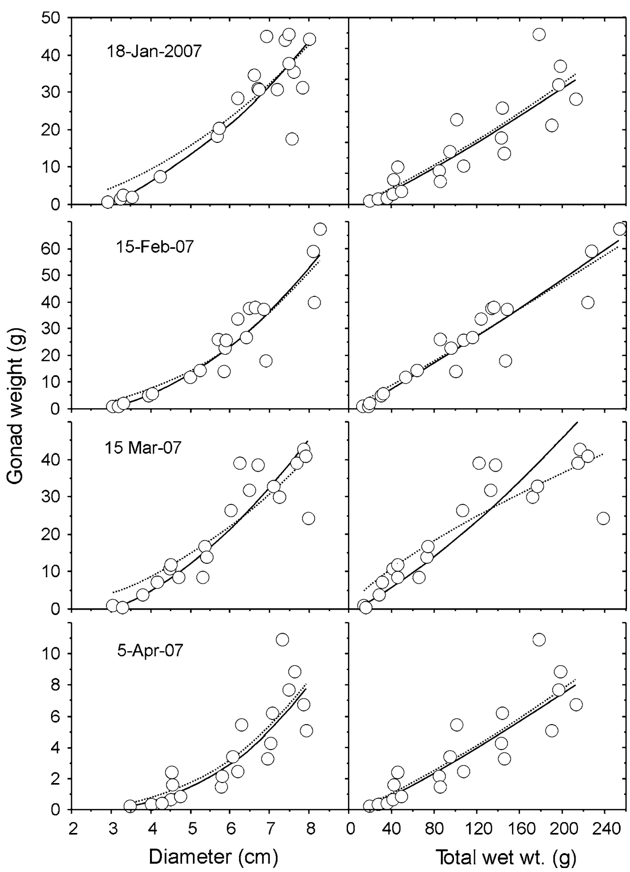

Fig. 5 Examples of *fitted lines* for *Strongylocentrotus purpuratus* gonad data using transformed (*solid line* Eq. 5) and untransformed (*dashed line* Eq. 4) data and both diameter and total wet weight

untransformed cross including going from positive to negative allometry (15 March 2007 total wet weight). The problems lie with how the variance of gonad weight changed with size and if there were outliers, as is the case for the 15 March sample. Outliers always were included in analysis. For purposes of gonad analyses, reducing such variability with a logarithmic transformation is appropriate and is our choice for analysis. Transformation also makes comparison easier because linear models can be used.

Five summary statistics of regressions using Eq. 5 are sufficient to permit other workers to compare their results with ours. These statistics are the means of X and Y, the standard error of the estimate, the coefficient of determination (R^2), and the slope of the regression, which is β. The following steps show how to reconstitute the important products and test for differences of slopes and intercepts. Notation follows Zar (1974). The standard error of the estimate

$$\mathrm{SE}_{\mathrm{est}} = \sqrt{\mathrm{MS}_{\mathrm{resid}}} \tag{12}$$

and so

$$\mathrm{SS}_{\mathrm{resid}} = (\mathrm{SE}_{\mathrm{est}})^2 \times (n-2) \tag{13}$$

The total sums of squares $\mathrm{SS}_{\mathrm{tot}} = \mathrm{SS}_{\mathrm{resid}}/(1-R^2)$ (14)

and so the sums of squares for the regression

$$\mathrm{SS}_{\mathrm{reg}} = \mathrm{SS}_{\mathrm{tot}} - \mathrm{SS}_{\mathrm{resid}}. \tag{15}$$

To compare samples, one also needs the means for X (ln(Dia − 0.7) or ln(Total − 0.5)) and Y (lnGonad). Slopes, β, are compared with a t-test

$$t = \frac{\beta_1 - \beta_2}{s_{\beta_1 - \beta_2}} \tag{16}$$

where

$$s_{\beta_1-\beta_2} = \sqrt{\frac{\left(s^2_{y\cdot x}\right)_p}{\left(\sum x^2\right)_1} + \frac{\left(s^2_{y\cdot x}\right)_p}{\left(\sum x^2\right)_2}}. \tag{17}$$

The pooled residual mean square is

$$\left(s^2_{y\cdot x}\right)_p = \frac{(SS_{\mathrm{resid}})_1 + (SS_{\mathrm{resid}})_2}{(DF_{\mathrm{resid}})_1 + (DF_{\mathrm{resid}})_2}. \tag{18}$$

It also is necessary to know the sums of squares of x and cross products xy, for each sample,

$$\sum x^2 = \frac{\left(\sum xy\right)^2}{SS_{\mathrm{reg}}} \tag{19}$$

and

$$\sum xy = \frac{SS_{\mathrm{reg}}}{b}. \tag{20}$$

The calculations for t can now be completed and the test has $n_1 + n_2 - 4$ degrees of freedom.

If slopes are judged to be different, this is the end of the analysis and separate regressions would be used for the two samples that include different slopes and intercepts. If, on the other hand, slopes are judged to be equal, analysis proceeds to a test for equality of intercepts.The first step is calculation of a common or weighted slope

$$\beta_c = \frac{(\sum xy)_1 + (\sum xy)_2}{(\sum x^2)_1 + (\sum x^2)_2} \quad (21)$$

The calculation of a t-value also requires a residual sum of squares for the common regression

$$\left(s^2_{y \cdot x}\right)_c = \frac{(SS_{\text{tot}})_1 + (SS_{\text{tot}})_2 - \left((\sum xy)_1 + (\sum xy)_2\right)^2 \Big/ \left((\sum x^2)_1 + (\sum x^2)_2\right)}{n_1 + n_2 - 3} \quad (22)$$

and so

$$t = \frac{\left(\overline{Y}_1 - \overline{Y}_2\right) - b_c\left(\overline{X}_1 - \overline{X}_2\right)}{\sqrt{\left(s^2_{y \cdot x}\right)_c \left[\frac{1}{n_1} + \frac{1}{n_2} + \frac{\left(\overline{X}_1 - \overline{X}_2\right)^2}{(\sum x^2)_1 + (\sum x^2)_2}\right]}} \quad (23)$$

with $n_1 + n_2 - 3$ degrees of freedom. An EXCEL file is available as an electronic supplement (Online Resource 1) to do these calculations with the summary statistics.

A comparison of results using the summary statistics compared with raw data for the 12 June 2007 from Gregory Point and the 8 June 1985 sample from Sunset Bay (Table 4) shows that the results are identical. Table 4B first shows the test for the equality of slopes by a t-test followed by the test for equality of intercepts. Table 4C is an ANCOVA (GLM, SYSTAT 2004) using raw data first testing for the significance of the interaction term of Sample × ln(Dia − 0.7) followed by a repeat of the analysis without interaction. The P-values are the same for the two analyses.

For comparison with analyses using Equations 4 and 5, a mean gonad index (Eq. 1) was calculated for each of the 31 monthly samples from Gregory Point using gonad and total wet weight (Fig. 6). The annual cycle is clear and shows differences across years and so discerning a pattern was not sensitive to violating the assumptions of the allometric exponent $\beta = 1.0$ and the correction for gonad development starting at $C = 0$. The gonad index, GI, was always above the other two measures, which is reasonable because the monthly total weights ranged from 110 to 131 g and so were always greater than 60 g. Differences, however, were greatest when gonads were large and smallest when gonads were small such as in April 2007 and 2008. An important point is that comparing the GI results with other studies or across years cannot be done because of the allometric problems presented here. The differences

Table 4 Comparison of samples from Gregory Point and Sunset Bay using summary statistics (A and B) and ANCOVA using raw data (C)

Sample	N	Mean X	Mean Y	R^2	SE_{est}	Slope b
A. Summary statistics						
Greg. Pt 12-Jun-07	20	1.679126	1.868634	0.928457	0.362400	4.000330
SS Bay 8-Jun-85	14	1.482973	0.612659	0.965010	0.388767	3.547010

Test	t	df	P	
B. Analysis				
Equality of slopes	1.3816	30	**0.1773**	Common slope = 3.694488
Equality of elevations	3.9230	31	**0.0005**	

Source	SS	df	MS	F-ratio	P
C. ANCOVA with raw data					
First a test of the interaction term Sample × ln(Dia − 0.7)					
ln(Dia − 0.7)	73.6772	1	73.6772	529.0769	<0.0001
Sample	0.0188	1	0.0188	0.1352	0.7157
Sample × ln(Dia − 0.7)	0.2658	1	0.2658	1.9087	**0.1773**
Error	4.1777	30	0.1393		
The P-value indicates that slopes should be considered equal so analysis is repeated without the interaction term Sample × ln(Dia − 0.7) to test for equality of elevations					
ln(Dia − 0.7)	80.4339	1	80.4339	561.1475	<0.0001
Sample	2.2060	1	2.2060	15.3899	**0.0005**
Error	4.4435	31	0.1433		

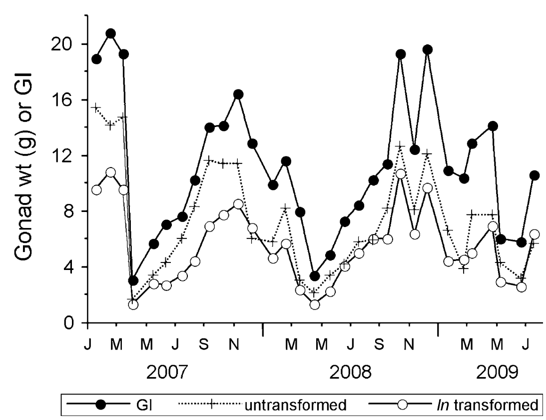

Fig. 6 Comparison of the gonad index, GI, with gonad weights of 60 g individuals using ln-transformed (Eq. 5) and untransformed (Eq. 4) data for analysis

shown by comparing the GI measures with a fixed size at 60 g illustrate what would happen if the Gregory Point data would be compared with another site where urchins were smaller but had gonad development that was the same as at

Table 5 Summary statistics for Gregory Point necessary to make comparisons with other data sets

ln(Dia − 0.7)						ln(Total − 0.5)			
Date	Mean X	Mean Y	R^2	SE_{est}	β	Mean X	R^2	SE_{est}	β
18-Jan-07	1.623894	2.780484	0.932627	0.345794	3.163906	4.508816	0.939573	0.327483	1.278657
15-Feb-07	1.561315	2.716974	0.941687	0.306905	3.309339	4.358372	0.938885	0.314193	1.334371
15-Mar-07	1.567001	2.618474	0.845128	0.509876	3.334608	4.364538	0.851047	0.500038	1.286367
5-Apr-07	1.623559	0.863748	0.859769	0.430335	3.552174	4.441182	0.873723	0.408364	1.403086
16-May-07	1.533483	1.327920	0.951083	0.348670	3.524766	4.312382	0.951604	0.346808	1.460516
12-Jun-07	1.679126	1.868634	0.928457	0.362400	4.000330	4.629693	0.913495	0.398497	1.631903
16-Jul-07	1.627732	1.757484	0.777751	0.622746	3.115279	4.514899	0.760028	0.647099	1.202826
13-Aug-07	1.606500	1.977332	0.782872	0.603856	3.311147	4.454059	0.780529	0.607105	1.273040
10-Sep-07	1.658567	2.599026	0.946835	0.277084	3.273598	4.608798	0.953990	0.257766	1.316229
9-Oct-07	1.614310	2.461821	0.700853	0.520154	2.639975	4.490030	0.740622	0.484346	1.060202
9-Nov-07	1.660099	2.636616	0.816147	0.287834	2.458353	4.480116	0.789045	0.308320	0.897178
9-Dec-07	1.610656	2.351104	0.841512	0.385011	2.851877	4.456740	0.822600	0.407334	1.100167
20-Jan-08	1.683387	2.293473	0.921631	0.296994	3.353287	4.669933	0.925336	0.289889	1.400881
18-Feb-08	1.700668	2.479511	0.844502	0.384580	3.017307	4.689144	0.857258	0.368468	1.231660
17-Mar-08	1.680871	1.915570	0.904342	0.414046	4.677095	4.633069	0.926954	0.361814	1.834413
16-Apr-08	1.730519	1.249900	0.726963	0.454827	3.551326	4.742478	0.761602	0.424998	1.451737
20-May-08	1.626549	1.375538	0.820471	0.421331	3.279435	4.485397	0.830888	0.408925	1.306484
18-Jun-08	1.661508	1.961731	0.976128	0.131924	2.730618	4.576119	0.974952	0.135133	1.062926
17-Jul-08	1.693622	2.164595	0.781410	0.268673	2.345807	4.658926	0.796040	0.259526	0.936657
17-Aug-08	1.710172	2.403011	0.820292	0.275615	2.436287	4.701094	0.828703	0.269088	0.983299
16-Sep-08	1.708334	2.509199	0.860947	0.276888	2.857314	4.708833	0.892721	0.243204	1.136685
14-Oct-08	1.713886	3.050741	0.892622	0.191308	2.650083	4.711072	0.892175	0.191706	1.016754
11-Nov-08	1.709409	2.577223	0.842714	0.358678	2.868121	4.718490	0.824848	0.378502	1.114566
9-Dec-08	1.650806	2.886705	0.913871	0.259206	3.219133	4.544310	0.919955	0.249882	1.227161
22-Jan-09	1.639816	2.189413	0.906680	0.411383	3.858299	4.528638	0.878260	0.469867	1.567373
23-Feb-09	1.660852	2.232215	0.842950	0.388576	3.575993	4.570397	0.843234	0.388225	1.373697
10-Mar-09	1.647363	2.364587	0.908927	0.393975	3.991557	4.524235	0.878093	0.455815	1.535567
24-Apr-09	1.724087	2.755876	0.865167	0.288380	3.079650	4.742094	0.870894	0.282189	1.160568
10-May-09	1.722779	1.920245	0.905231	0.237335	3.103343	4.753133	0.921787	0.215609	1.176879
23-Jun-09	1.647015	1.613116	0.934491	0.268021	3.353655	4.514755	0.932933	0.271190	1.334615
20-Jul-09	1.709943	2.421587	0.883836	0.224695	2.247265	4.688953	0.867086	0.240349	0.922270

Mean of Y is ln gonad wet weight and mean of X is for ln(Dia − 0.7 cm) and ln(Total wet weight − 0.5). Natural logarithms are used. The large number of significant figures are important when they are used for comparison with other data sets

Gregory Point. Sea urchins at Gregory Point would be judged to be more productive whereas they really would be just larger individuals.

Discussion

We have shown that simple assumptions about the allometry of gonad development in a sea urchin make the gonad index, GI (Eq. 1) suspect for any application other than description of the reproductive cycle, which has been recognized by others (Marsh and Watts 2007). Because gonads begin to develop at a test diameter greater than zero, an additional parameter, C, is required in analysis. Estimation of this parameter from dissection data has large standard errors (Table 2) and so the values presented here for *S. purpuratus* must be viewed as provisional. The estimate of $C = 0.7$ cm for test diameter when gonads begin to develop, however, is similar to the estimates of 0.7–0.8 cm for *S droebachiensis* (O. Ellers, personal communication). To our knowledge, there are no histological studies that would provide clearer evidence for when gonads in *S. purpuratus* begin to develop.

Data from Gregory Point, Oregon, showed that the allometric exponent β was not usually equal to 1.0 when total wet weight was used as the independent variable or equal to 3.0 when diameter was used (Fig. 3). Furthermore, the exponent changed during the year so a single value could not be used. Individual regressions were required to describe each sample (Table 5). If a research goal includes comparing size-specific gonad development or differences between sites or times (e.g., Lessios 1981; Boivin et al. 1986; Lawrence and Byrne 1994; Lozano et al. 1995; Lester et al. 2007), use of the simple GI runs the risk of producing errors that may be serious (c.f. Beaupre and Dunham 1995).

A solution we have presented here is to compare samples by selecting a specific size, either diameter or total weight, and estimating gonad size although other measures of total and gonad size could be used such as volume or dry weights. We selected 5 cm test diameter and 60 g total wet weight because these are values found in samples over a very wide geographic range for *Strongylocentrotus purpuratus*; individuals with diameters ≥8 cm are not present at most sites along the coast (Ebert 2010a). Comparing samples in other studies can be done using the five summary statistics we have provided; statistical comparisons of gonad index, GI, values across studies cannot be done when different sizes of individuals are being compared and, given the problems presented here, should not be done within a study.

Using a wide size range of individuals in a study has further benefits. Comparisons of life histories of sea urchins are difficult. In the past, when the Brody–Bertalanffy growth model still seemed appropriate (Ebert 1975), a comparison of the parameter K and the instantaneous mortality rate, M, was reasonable. Indeed, if growth data are forced to fit the Brody–Bertalanffy equation (Ebert 2007) comparisons still can be done in this manner. If a different growth model is used, however, such as the Richards or Tanaka, there no longer is a single parameter that can represent growth. An alternative is to use production and biomass estimates (Ebert 2010b), an approach that requires estimating age-specific spawn biomass. Gonad data gathered for all available sizes and at times of maximum and minimum gonad size can be used to calculate size-specific spawn mass and, with growth estimates, age-specific spawn mass (e.g., Ebert 2008).

Our recommendations for studies where body components are to be compared are as follows:

1. Gather a wide range of sizes including small individuals rather than concentrating on a restricted size range. Work of dissection is the same and information content is much greater.
2. Use the allometry model with an adjustment for size when gonads begin to develop (Eq. 4).
3. Publish the summary statistics.
4. Save the data in a form that can be shared because there is no substitute for the raw data.

Acknowledgments Dissections of sea urchins from Gregory Point were done by B. Miller. Those from Arena Cove, California, were done by M. Morris and N. Sandoval. Assistance with dissections from Yaquina Head, Oregon, was by C. Ebert. The manuscript benefited from comments made by 3 anonymous reviewers. Funding for this work was from the Ocean Sciences Division Biological Oceanography of the US National Science Foundation (grants OCE 84-01415 and OCE-0623934). We are grateful for all of this support.

References

Atchley WR, Gaskins CT, Anderson D (1976) Statistical properties of ratios. I. Empirical results. Syst Zool 25:137–148. doi:10.2307/2412740

Barker MF, Xu RA (1991) Population differences in gonad and pyloric caeca cycles of the New Zealand seastar *Sclerasterias mollis* (Echinodermata: Asteroidea). Mar Biol 108:97–103. doi:10.1007/BF01320228

Beaupre SJ, Dunham AE (1995) A comparison of ratio-based and covariance analyses of a nutritional data set. Funct Ecol 9:876–880

Bennett J, Giese AC (1955) The annual reproductive and nutritional cycles in two western sea urchins. Biol Bull 109:226–237

Bernard FR (1977) Fishery and reproductive cycle of the Red Sea urchin, *Strongylocentrotus franciscanus*, in British Columbia. J Fish Res Bd Can 34:604–610

Bigatti, G, Marzinelli EM, Cledón M, Penchaszadeh PE (2004) Gonadal cycle of *Pseudechinus magellanicus* (Philippi, 1857) (Echinoidea: Temnopleuridae) from Patagonia, Argentina. In: Heinzeller T, Nebelsick JH (eds) Echinoderms: München. Proceedings, 11th international echinoderm conference, A. A. Balkema, Leiden, The Netherlands, pp 11–14

Boivin Y, Larrivée D, Himmelman JH (1986) Reproductive cycle of the subarctic brooding asteroid *Leptasterias polaris*. Mar Biol 92:329–337. doi:10.1007/BF00392673

Bourgoin A, Guillou M (1990) Variations in the reproductive cycle of *Acrocinida brachiata* (Echinodermata: Ophiuroidea) according to environment in the Bay of Douarnenez (Brittany). J Mar Biol Ass UK 70:57–66. doi:10.1017/S0025315400034196

Byrne M (1992) Reproduction of sympatric populations of *Patiriella gunnii*, *P. calcar* and *P. exigua* in New South Wales, asterinid sea stars with direct development. Mar Biol 114:297–316. doi:10.1007/BF00349533

Byrne M, Morrice MG, Wolf B (1997) Introduction of the northern Pacific asteroid *Asterias amurensis* to Tasmania: reproduction and current distribution. Mar Biol 127:673–685. doi:10.1007/s002270050058

Byrne M, Andrew NL, Worthington DG, Brett PA (1998) Reproduction in the diadematoid sea urchin *Centrostephanus rodgersii* in contrasting habitats along the coast of New South Wales, Australia. Mar Biol 132:305–318. doi:10.1007/s002270050396

Cameron JL, Fankboner PV (1986) Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson) (Echinodermata: Holothuroidea). I. Reproductive periodicity and spawning behavior. Can J Zool 64:168–175. doi:10.1139/z86-027

Chadwick-Furman NE, Spiegel M, Nir I (2000) Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. Invert Biol 119:361–369

Chao S-M, Chen C-P, Alexander PS (1995) Reproductive cycles of tropical sea cucumbers (Echinodermata: Holothuroidea) in southern Taiwan. Mar Biol 122:289–295. doi:10.1007/BF00348942

Chen B-Y, Chen C-P (1992) Reproductive cycle, larval development, juvenile growth and population dynamics of *Patiriella psudoexigua* (Echinodermata: Asteroidea) in Taiwan. Mar Biol 113:271–280. doi:10.1007/BF00347281

Christians JK (1999) Controlling for body mass effects: is part-whole correlation important? Physiol Biochem Zool 72:250–253. doi:10.1086/316661

Costa PAS, Fernandes FC (1993) Reproductive cycle of *Loligo sanpaulensis* (Cephalopoda: Loliginidae) in the Cabo Frio region, Brazil. Mar Ecol Prog Ser 101:91–97

Damián GG, Zaixso HE, Tolosano JA (2009) Brooding of the sub-Antarctic heart urchin, *Abatus cavernosus* (Spatangoida: Schizasteridae), in southern Patagonia. Mar Biol 156:1647–1657. doi:10.1007/s00227-009-1200-7

de Vlaming V, Grossman G, Chapman F (1982) On the use of the gonosomatic index. Comp Biochem Physiol A 73:31–39. doi:10.1016/0300-9629(82)90088-3

Diez MJ, Lovrich GA (2010) Reproductive biology of the crab *Halicarcinus planatus* (Brachyura, Hymenosomatidae) in sub-Antarctic waters. Polar Biol doi:10.1007/s00300-009-0716-0

Ebert TA (1968) Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. Ecology 49:1075–1091. doi:10.2307/1934491

Ebert TA (1975) Growth and mortality of post-larval echinoids. Am Zool 15:755–775

Ebert TA (1998) An analysis of the importance of Allee effects in management of the red sea urchin *Strongylocentrotus franciscanus*. In: Mooi R, Telford M (eds) Echinoderms: San Francisco. Proceedings, 9th international echinoderm conference, A. A. Balkema, Brookfield, Vermont, pp 619–627

Ebert TA (2007) Growth and survival of postsettlement sea urchins. In: Lawrence JM (ed) Edible sea urchins: biology and ecology. Developments in aquaculture and fisheries science, vol 37. Elsevier, Amsterdam, pp 95–134

Ebert TA (2008) Longevity and lack of senescence in the red sea urchin *Strongylocentrotus franciscanus*. Exp Gerontol 43:734–738. doi:10.1016/j.exger.2008.04.015

Ebert TA (2010a) Demographic patterns of the purple sea urchin *Strongylocentrotus purpuratus* along a latitudinal gradient, 1985–1987. Mar Ecol Prog Ser 406:105–120. doi:10.3354/meps08547

Ebert TA (2010b) Potential use of production and biomass for life-history comparisons of sea urchins. Proceedings 13th international echinoderm conference, Hobart, Tasmania (in press)

Ebert TA, Russell MP (1994) Allometry and model II nonlinear regression. J Theor Biol 168:367–372

Ford CE (1964) Reproduction in the aggregating sea anemone *Anthopleura elegantissima*. Pac Sci 18:138–145

Giese AC (1966) On the biochemical constitution of some echinoderms. In: Boolootian RA (ed) Physiology of Echinodermata. Interscience Publishers (Wiley), New York, pp 757–796

Giese AC (1967) Changes in body-component indexes and respiration with size in the purple sea urchin *Strongylocentrotus purpuratus*. Physiol Zool 40:194–200

Giese AC, Greenfield L, Huang H, Farmanfarmaian A, Boolootian R, Lasker R (1959) Organic productivity in the reproductive cycle of the purple sea urchin. Biol Bull 116:49–58

Gonor JJ (1972) Gonad growth in the sea urchin, *Strongylocentrotus purpuratus* (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. J Exp Mar Biol Ecol 10:89–103. doi:10.1016/0022-0981(72)90095-0

Guettaf M, San Martin GA, Francour P (2000) Interpopulation variability of the reproductive cycle of *Paracentrotus lividus* (Echinodermata: Echinoidea) in the south-western Mediterranean. J Mar Biol Ass UK 80:899–907. doi:10.1017/S0025315400002885

Guillou M, Lumingas LJL (1998) The reproductive cycle of the 'blunt' sea urchin. Aquacul Internat 6:147–160. doi:10.1023/A:1009290307840

Guillou M, Lumingas LJL (1999) Variation in the reproductive strategy of the sea urchin *Sphaerechinus granularis* (Echinodermata: Echinoidea) related to food availability. J Mar Biol Ass UK 79:131–136. doi:10.1017/S0025315498000149

Gutt J, Gerdes D, Klages M (1992) Seasonality and spatial variability in the reproduction of two Antarctic holothurians (Echinodermata). Polar Biol 11:533–544. doi:10.1007/BF00237946

Hatfield EMC, Murray AWA (1999) Objective assessment of maturity in the Patagonian squid *Loligo gahi* (Cephalopoda: Loliginidae) from Falkland Islands waters. ICES J Mar Sci 56:746–756. doi:10.1006/jmsc.1999.0514

Hines AH (1979) Effects of thermal discharge on reproductive cycles in *Mytilus edulis* and *Mytilus californianus* (Mollusca, Bivalvia). Fish Bull 77:498–503

Hughes AD, Kelly MS, Barnes DKA, Catarino AI, Black KD (2006) The dual functions of sea urchin gonads are reflected in the temporal variations of their biochemistry. Mar Biol 148:789–798. doi:10.1007/s00227-005-0124-0

Kato S, Schroeter SC (1985) Biology of the red sea urchin, *Strongylocentrotus franciscanus*, and its fishery in California. Mar Fish Rev 47:1–20

Kelly MS, Owen PV, Pantazis P (2001) The commercial potential of the common sea urchin *Echinus esculentus* from the west coast of Scotland. Hydrobiol 465:85–94. doi:10.1023/A:1014553010711

Kirby S, Lamare MD, Barker MF (2006) Growth and morphometrics in the New Zealand sea urchin *Pseudechinus huttoni* (Echinoidea: Temnopleuridae). NZ J Mar Freshwater Res 40:413–428. doi:0028-8330/06/4003-0413

Kramer DE, Nordin DMA (1975) Physical data from a study of size, weight and gonad quality for the red sea urchin [*Strongylocentrotus franciscanus* (Agassiz)] over a one-year period. Fish Res Bd Can Manuscript Rpt Ser No 1372, 91 pp

Laegdsgaard P, Byrne M, Anderson DT (1991) Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *H. tuberculata* (Echinoidea) in New South Wales. Mar Biol 110:359–374. doi:10.1007/BF01344355

Lau DCC, Lau SCK, Qian P-Y, Qiu J-W (2009) Morphological plasticity and resource allocation in response to food limitation and hyposalinity in a sea urchin. J Shellfish Res 28:383–388

Lawrence JM (1973) Level, content, and caloric equivalents of the lipid, carbohydrate, and protein in the body components of *Luidia clathrata* (Echinodermata: Asteroidea: Platyasterida) in Tampa Bay. J Exp Mar Biol Ecol 11:263–274. doi:10.1016/0022-0981(73)90026-9

Lawrence JM, Byrne M (1994) Allocation of resources to body components in *Heliocidaris erythrogramma* and *Heliocidaris tuberculata* (Echinodermata: Echinoidea). Zool Sci 11:133–137

Lawrence JM, Lawrence AL, Holland ND (1965) Annual cycle in the size of the gut of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson). Nature 205:1238–1239. doi:10.1038/2051238a0

Lefebvre A, Davoult D, Gentil F, Janquin MA (1999) Spatio-temporal variability in the gonad growth of *Ophiothrix fragilis* (Echinodermata: Ophiuroidea) in the English Channel and estimation of carbon and nitrogen outputs towards the pelagic system. Hydrobiologia 414:25–34. doi:10.1023/A:1003827532385

Lessios HA (1981) Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama. J Exp Mar Biol Ecol 50:47–61. doi:10.1016/0022-0981(81)90062-9

Lester SE, Gaines SD, Kinlan BP (2007) Reproduction on the edge: large-scale patterns of individual performance in a marine invertebrate. Ecology 88:2229–2239. doi:10.1890/06-1784.1

Lewis JB (1958) The biology of the tropical sea urchin *Tripneustes esculentus* Leske in Barbados, British West Indies. Can J Zool 36:607–621

Lewis JB (1966) Growth and breeding in the tropical echinoid *Diadema antillarum* Phillipi. Bull Mar Sci 16:151–158

Lewis JB, Storey GS (1984) Differences in morphology and life history traits of the echinoid *Echinometra lucunter* from different habitats. Mar Ecol Prog Ser 15:207–211

Liermann M, Steel A, Rosingard M, Guttorp P (2004) Random denominators and the analysis of ratio data. Envir Ecol Stat 11:55–71. doi:10.1023/B:EEST.0000011364.71236.f8

Liu JH (1994) The ecology of the Hong Kong limpets *Cellana grata* (Gould 1859) and *Patelloida pygmaea* (Dunker 1860): reproductive biology. J Moll Stud 60:97–111

Lozano J, Galera J, López S, Turon X, Palacin C, Morera G (1995) Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. Mar Ecol Prog Ser 122:179–191. doi:10.3354/meps122179

MacCord FS, Ventura CRR (2004) Reproductive cycle of the endemic cassiduloid *Cassidulus mitis* (Echinoidea: Cassiduloida) on the Brazilian coast. Mar Biol 145:603–612. doi:10.1007/s00227-004-1344-4

Marsh AG, Watts SA (2007) Biochemical and energy requirements of gonad development. In: Lawrence JM (ed) Edible sea urchins: biology and ecology. Developments in aquaculture and fisheries science, vol 37. Elsevier, Amsterdam, pp 35–53

McCarthy DA, Young CM (2002) Gametogenesis and reproductive behavior in the echinoid *Lytechinus variegatus*. Mar Ecol Prog Ser 233:157–168. doi:10.3354/meps233157

McPherson BF (1965) Contributions to the biology of the sea urchin *Tripneustes ventricosus*. Bull Mar Sci 15:228–244

Moore HB, Jutare T, Jones JA, McPherson BF, Roper CFE (1963) A contribution to the biology of *Tripneustes esculentus*. Bull Mar Sci Gulf Carib 13:267–281

Nicastro KR, Zardi GI, McQuaid CD (2010) Differential reproductive investment, attachment strength and mortality of invasive and indigenous mussels across heterogeneous environments. Biol Invasions doi:10.1007/s10530-009-9619-9

Nichols D, Barker MF (1984) Reproductive and nutritional periodicities in the starfish, *Marthasterias glacialis*, from Plymouth Sound. J Mar Biol Ass UK 64:461–470. doi:10.1017/S0025315400030137

Nojima S (1979) Ecological studies of a sea star, *Astropecten latespinosus* Meissner. I. Survivorship curve and life history. Publ Amakusa Mar Biol Lab 5:45–65

Olive PJW, Porter JS, Sandeman NJ, Wright NH, Bentley MG (1997) Variable spawning success of *Nepthys hombergi* (Annelida: Polychaeta) in response to environmental variation a life history homeostasis? J Exp Mar Biol Ecol 215:247–268. doi:10.1016/S0022-0981(97)00047-6

Ong Che RG, Gomez ED (1985) Reproductive periodicity of *Holothuria scabra* jaeger at Calatagan, Batangas, Philippines. Asian Mar Biol 2:21–30

Packard GC, Boardman TJ (1987) The misuse of ratios to scale physiological data that vary allometrically with body size. In: Feder ME, Bennett AF, Burggren WW, Huey RB (eds) New directions in ecological physiology. Cambridge University Press, Cambridge, pp 216–239

Packard GC, Boardman TJ (1999) The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? Comp Biochem Physiol A 122:37–44

Pearse JS (1968) Patterns of reproductive periodicities in four species of Indo-Pacific echinoderms. Proc Indian Acad Sci B 68:247–279

Pérez-González R, Puga-López D, Castro-Longoria R (2009) Ovarian development and size at sexual maturity of the Mexican spiny lobster *Panulirus inflatus*. NZ J Mar Freshwater Res 43:163–172. doi:10.1080/00288330909509990

Quackenbush LS, Herrnkind WF (1981) Regulation of molt and gonadal development in the spiny lobster, *Panulirus argus* (Crustacea: Palinuridae): effect of eyestalk ablation. Comp Biochem Physiol A 69:523–627. doi:10.1016/0300-9629(81)93014-0

Raubenheimer D, Simpson SJ (1992) Analysis of covariance: an alternative to nutritional indices. Entomol Exp Appl 62:221–231

Raymond BG, Scheibling RE (1987) Recruitment and growth of the sea urchin *Strongylocentrotus droebachiensis* (Muller) following mass mortalities off Nova Scotia, Canada. J Exp Mar Biol Ecol 108:31–54. doi:10.1016/0022-0981(87)90129-8

Robb RC (1929) On the nature of hereditary size-limitation. II. The growth of parts in relation to the whole. Brit J Exp Bio 6:311–324

Russell MP (1998) Resource allocation plasticity in sea urchins: rapid, diet induced, phenotypic changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller). J Exp Mar Biol Ecol 220:1–14. doi:10.1016/S0022-0981(97)00079-8

Selden R, Johnson AS, Ellers O (2009) Waterborne cues from crabs induce thicker skeletons, smaller gonads and size-specific changes in growth rate in sea urchins. Mar Biol 156:1057–1071. doi:10.1007/s00227-009-1150-0

SYSTAT (2004) SYSTAT 11. SYSTAT Software, Inc, Richmond, CA

Town JC (1980) Movement, morphology, reproductive periodicity, and some factors affecting gonad production in the seastar, *Asterstole scabra* (Hutton). J Exp Mar Biol Ecol 44:111–132. doi:10.1016/0022-0981(80)90149-5

Vadas RL Sr, Beal B, Dowlin T, Fegley JC (2000) Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. Aquaculture 182:115–135. doi:10.1016/S0044-8486(99)00254-9

Vernon JD, McClintock JB, Hopkins TS, Watts SA, Marion KR (1993) Reproduction of *Clypeaster ravenelii* (Echinodermata: Echinoidea) in the northern Gulf of Mexico. Invert Reprod Dev 24:71–78

Zar JH (1974) Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ