

Abstract—Body size at gonadal maturity is described for females of the slipper lobster (*Scyllarides squammosus*) (Scyllaridae) and the endemic Hawaiian spiny lobster (*Panulirus marginatus*) (Palinuridae) based on microscopic examination of histological preparations of ovaries. These data are used to validate several morphological metrics (relative exopodite length, ovigerous condition) of functional sexual maturity. Relative exopodite length (“pleopod length”) produced consistent estimates of size at maturity when evaluated with a newly derived statistical application for estimating size at the morphometric maturation point (MMP) for the population, identified as the midpoint of a sigmoid function spanning the estimated boundaries of overlap between the largest immature and smallest adult animals. Estimates of the MMP were related to matched (same-year) characterizations of sexual maturity based on ovigerous condition—a more conventional measure of functional maturity previously used to characterize maturity for the two lobster species. Both measures of functional maturity were similar for the respective species and were within 5% and 2% of one another for slipper and spiny lobster, respectively. The precision observed for two shipboard collection series of pleopod-length data indicated that the method is reliable and not dependent on specialized expertise. Precision of maturity estimates for *S. squammosus* with the pleopod-length metric was similar to that for *P. marginatus* with any of the other measures (including conventional evidence of ovigerous condition) and greatly exceeded the precision of estimates for *S. squammosus* based on ovigerous condition alone. The two measures of functional maturity averaged within 8% of the estimated size at gonadal maturity for the respective species. Appendage-to-body size proportions, such as the pleopod length metric, hold great promise, particularly for species of slipper lobsters like *S. squammosus* for which there exist no other reliable conventional morphological measures of sexual maturity. Morphometric proportions also should be included among the factors evaluated when assessing size at sexual maturity in spiny lobster stocks; previously, these proportions have been obtained routinely only for brachyuran crabs within the Crustacea.

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Relative pleopod length as an indicator of size at sexual maturity in slipper (*Scyllarides squammosus*) and spiny Hawaiian (*Panulirus marginatus*) lobsters

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Estimates of body size and age at sexual maturity provide key information for stock assessments and hence for managing sustainable fisheries. Characterizations of size at maturity are relatively straightforward in lobsters and most other crustaceans. One presently accepted standard is to regress percentage mature against classes of some body size metric and to fit a logistic model to predict the size class in which 50% of the population is mature. A necessary prerequisite is accurate data on the maturation state of individuals. In spiny lobsters of the family Palinuridae, female maturation is usually deduced from “berried” (ovigerous) condition (Groeneveld and Melville-Smith, 1994), the presence of external morphological indicators such as changes in the number of pleopod setae (Gregory and Labisky, 1981; Montgomery, 1992), relative lengths of abdominal and thoracic segments (Jayakody, 1989), or proportional lengths of segments of walking or egg-bearing appendages at the pubertal molt (George and Morgan, 1979; Grey, 1979; Juinio, 1987; Plaut, 1993; Evans et al., 1995; Hogarth and Barratt, 1996; Minagawa and Higuchi, 1997). A major complication arises, however, when the percentage mature within size classes cannot be accurately described. Such is the case for *Scyllarides squammosus*, a species of slipper lobster (family Scyllaridae) that prior to closure of the fishery in 2000

had become an increasingly important target of the Northwestern Hawaiian Island (NWHI) commercial trap fishery. In *S. squammosus*, unberried but mature females are indistinguishable, based on gross external morphology, from immature females. In this species, the additional variance introduced by combining falsely classified “immature” with truly immature females inflates requisite sample sizes enough (given the sampling effort feasible on annual research surveys) to prevent characterization of possible changes in size at maturity with data pooled from less than several surveys. Combining unberried adults with true immature individuals also introduces an overestimation bias (DeMartini et al., 2003).

To date only one study has provided a description of the use of a morphological measure of maturity in a slipper lobster (Hossain, 1978). Morphology-based maturity measures have been described for numerous spiny lobsters of the genus *Panulirus*, but such measures for the endemic Hawaiian spiny lobster (*Panulirus marginatus*) have not been fully described (Prescott, 1984).

Our objectives are to describe the development and use of an external body metric for accurately and precisely characterizing body size at morphological (functional) sexual maturity in female *Scyllarides squammosus*. We likewise use this external metric

to estimate size at maturity of females of the Hawaiian spiny lobster, for which functional maturity can be accurately described by using a combination of other, more apparent external features. We also estimate body size at gonadal maturity by microscopic examination of histological preparations of ovaries of each species and use these results to validate the functional maturity characterizations. We contrast the benefits of the different approaches for estimating functional maturity in these two lobsters and discuss the potential importance of applying efficient measures of maturation for managing the NWHI lobster fishery.

Materials and methods

Specimen collection

A research vessel was used to set and retrieve lobster traps. All specimens of spiny lobster used in this study were taken from Necker Bank surrounding Necker Island (23°34'N, 164°42'W), NWHI. All the slipper lobsters used were taken from Maro Bank, located about 600 km to the northwest of Necker at 25°25'N, 170°35'W. Lobsters were caught from bank terraces at median depths of 15 fm (slipper lobsters, Maro) and 17 fm (spiny lobsters, Necker) with molded plastic (Fathoms Plus®, San Diego, CA) traps baited with 1 kg of mackerel (*Scomber japonicus*) and left for a standard (overnight) soak.

Shipboard processing

All specimens were processed alive within minutes of trap retrieval. Tail width (TW), as defined for slipper lobster by DeMartini and Williams (2001) and for Hawaiian spiny lobster by DeMartini et al. (2003), was measured with 0.1 mm accuracy. Berried females were scored by egg-development stage with a gross visual proxy (brooded eggs noted as either orange or brown in color to the unaided eye). Female spiny lobsters were scored by the presence or absence and by condition ("smooth"=unused, "rough"=partly used) of spermatophoric (sperm) mass (Matthews, 1951; Berry and Heydorn, 1970) on the sternum. Female *S. squamosus* in almost all cases lack a sperm mass and the presence-absence of this feature provides no useful information. In 1998–2000, ovaries were dissected from a maximum of two living specimens for each 1-mm TW class of the two species and fixed in 10% (sea water buffered) formalin for subsequent histological analyses. Egg-bearing "tails" (abdominal segments) were flash-frozen at –20°C.

During 1997–99, pleopods of each species were measured aboard ship to evaluate measurement accuracy under field conditions. Maxima of 10 live individuals per 1-mm TW class of each species were measured as described below. Two independent measurements of each specimen were made by each of two measurers (one inexperienced and one experienced). In 2000–01, pleopods for a larger series of morphometrics were simi-

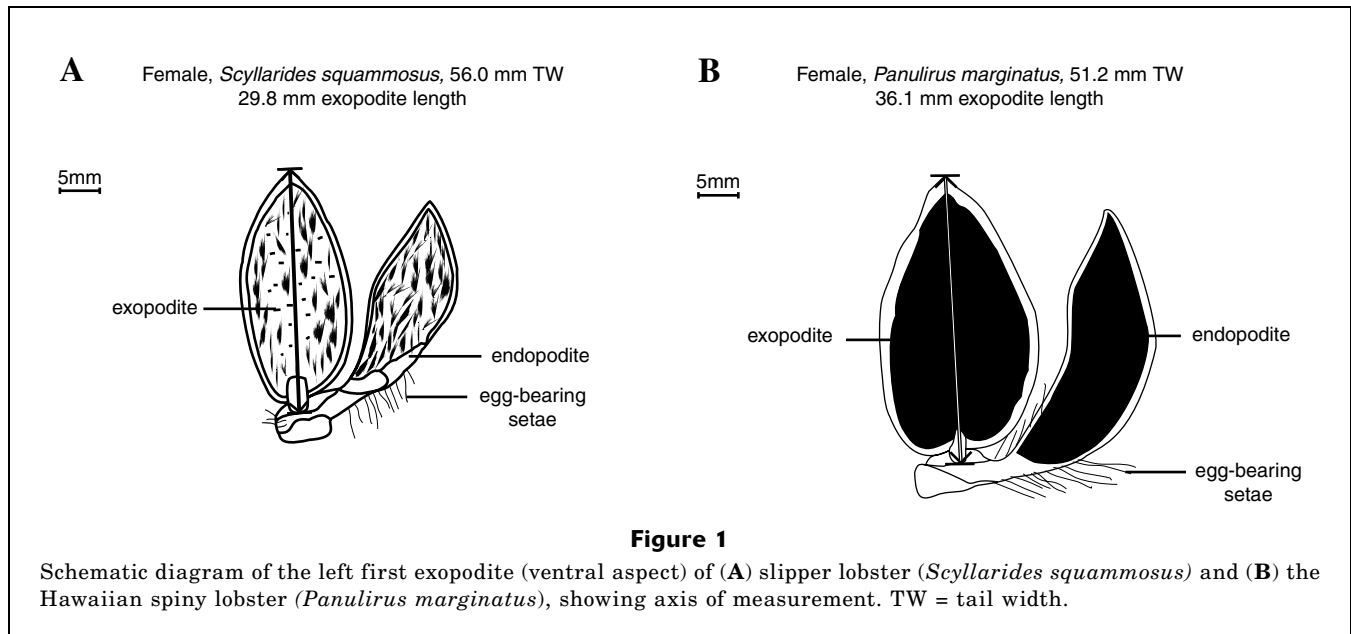
larly measured aboard ship to evaluate production-scale numbers (500–1000 specimens per species on each cruise) based on a single measurement per specimen taken by one measurer.

Laboratory measurements

Beginning with specimens collected in 2000, the lengths of exopodites on first pleopods were measured for a representative sample of berried and unberried tails of each species, after the tails were thawed overnight in a refrigerator at 3°C. Preliminary observations indicated that the first pleopod was disproportionately large in berried females; measurements of the first pleopod of all (berried and unberried) females moreover were the most precise, i.e. the measurements were more likely to be obtained again—probably because the first pleopod was the easiest to measure. The straightline distance between base and tip of exopodite on the first pleopod (exopodite length=EL) was measured with dial calipers to 0.01 mm. An analogous measurement of exopodite width (EW) was taken perpendicular to the EL axis at the structure's widest point. The left exopodite in ventral aspect (Fig. 1) was routinely measured because the ventral aspect was easier to measure for live animals aboard ship. Measurements of the right exopodite (of the same specimen) in dorsal aspect were taken for a range of body sizes to evaluate the possible influence of aspect (dorsal vs. ventral) or body side (left vs. right) on the measurement that was taken. Replicate measurements (independent, with calipers reset to zero between measurements) were used to assess inter-measurer and inherent measurement error. Formalized ovaries were weighed (blotted damp-dry) to the nearest 0.01 g after fixation for at least a month.

Histological validation

Fixed ovary specimens of each species were dehydrated, imbedded, and sectioned by using standard techniques, and were stained with hematoxylin and counter-stained with eosin to differentiate protein and yolk materials within oocytes. Histological slides were viewed under a compound microscope at 150× magnification. For each specimen, the diameters (average of major and minor axis) were measured for 10 oocytes (randomly chosen) within the largest size class of oocytes present. The median diameter was used to characterize oocyte size for that specimen; the median diameter based on 10 measurements yielded CVs ($100\% \times \text{standard error}/\text{mean}$) <10% (DeMartini et al., 2003). Developmental staging followed Minagawa (1997) and Minagawa and Sano (1997): females were scored as mature 1) if unberried in developing or ripe ovarian stages II and III, respectively; 2) if berried in ripe and redeveloping stages IV and V, respectively; or 3) if recently spent (stage VI) with heavily setose pleopods (*P. marginatus* only). Inactive females in stage I were scored as immature. A gonad index, calculated as $GI = (OW \times 10^5 / TW^3)$, where OW = ovary weight in g, was used to complement his-

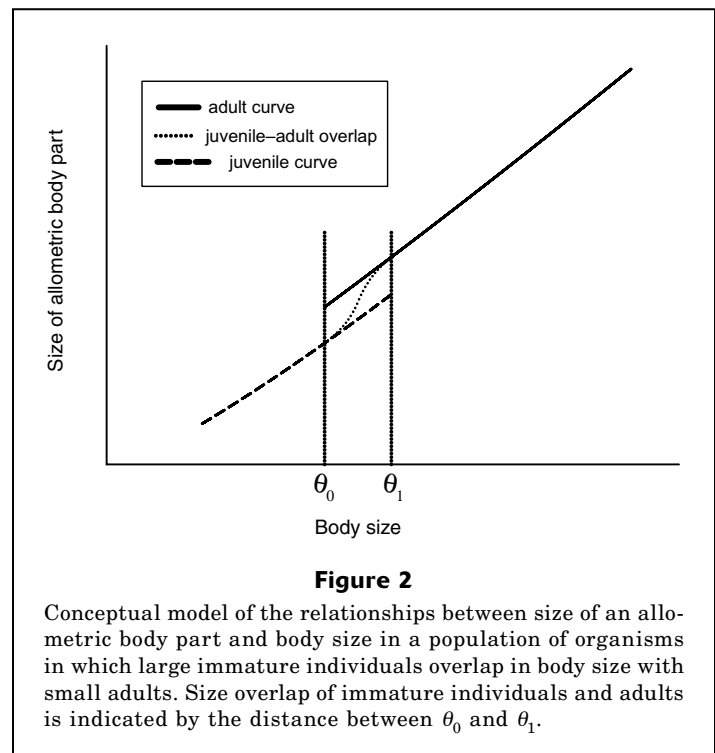


tological scores in assessing gonadal maturity (Minagawa and Sano, 1997). Gonadal maturity was used as a means of validating, as well as referencing, estimates of size at functional maturity (Ennis, 1984).

Statistical analyses

Data for EL and EW (as response variables) and TW (regressor) for the same specimen were first plotted for all specimens of each species. Preliminary evaluations of these data (both raw and log-transformed) with least-squares linear regression (REG procedure; SAS vers. 8, SAS Institute, Inc., Cary, NC) indicated allometric relationships for which double-log functions provided approximate fits. Identification of join points by iteration based on minimizing the total residual sums of squares of pairs of joined regression equations (Somerton, 1980), however, resulted in linear spline fits that, although significant, had obviously nonrandom residuals. Simple linear fits with log-log plots, however, were useful for selecting the most appropriate metric: the regressions of EW on TW, qualitatively similar to those for EL regressed on TW, had consistently lower r^2 values, likely because pleopod width was more difficult to measure than pleopod length. The EL metric was therefore chosen for all further analyses.

Because lobsters, like most biological populations, are composed of individuals that differ in the size at which first maturity occurs, we fitted a curve to the EL-TW relation that included a sigmoid segment bridging the region between the estimated sizes of the smallest adults (θ_0) and the largest immature individuals (θ_1) (Fig. 2). The curve was fitted by using iterative reweighted least



squares (S-Plus 6 for Windows, Insightful Corporation, Seattle, WA; Ratkowsky, 1983) with appropriate weights to standardize the variance (Appendix). The morphometric maturation point, hereafter referred to as the MMP, was estimated at the inflection ($[\theta_0 + \theta_1]/2$) of the sigmoid segment of the curve. This inflexion point represents the body size at which we expect 50% of the lobsters to become sexually mature (median size at attainment of

maturity). Confidence bounds on the MMP were estimated by using the studentized bootstrap method (Davison and Hinkley, 1997) with 1000 iterations.

In order to characterize median body size at gonadal maturity, the percentage mature per 5-mm TW class, deduced from viewing histological preparations of ovaries, was fitted to the conventional (2-parameter) logistic model,

$$P_x = 100 / \{1 + \exp[-a(TW - b)]\},$$

where P_x = percent mature at $TW = x$;
 a and b are unknown constants; and
 TW = tail width in mm.

To similarly estimate median size at maturity based on gross external characteristics, the percentage mature per 5-mm TW class was fitted to a 3-parameter logistic model for *Scyllarides squammosus* and to the conventional 2-parameter logistic for *Panulirus marginatus*. For *S. squammosus*, percentage maturity per 5-mm TW class was estimated by fitting the 3-parameter logistic equation,

$$P_x = 100a / \{1 + \exp[(4b/a)(c - TW)]\},$$

where a = the asymptotic proportion berried;
 b = the slope of the logistic function at the inflection point; and
 $c = TW_{50}$ is the tail width at the inflection point (size at 50% of asymptote).

This function has been fully described for estimating percentage maturity based on incidence of ovigerous females in *S. squammosus*; the extra parameter is needed to fit an asymptote to the sigmoidal function at a value less than 100% (DeMartini et al., 2002). Parameters of the various models were estimated by using the maximum-likelihood nonlinear curve fitting procedure SAS NLIN; all nonlinear regressions were weighted by the square root of sample sizes.

The body size at which 50% of the population was estimated as mature (hereafter referred to as TW_{50}) was compared for 1) TW_{50} based on the relative incidence of berried individuals within the female population (both species), adjusted for the co-presence of a sperm mass (*P. marginatus* only), 2) TW_{50} estimated from histological evidence (both species), and 3) the MMP of the allometric EL-to-TW relation (both species). Estimates were compared graphically among methods for each species.

Analyses of pleopod-based maturity followed a series of evaluations of pleopod characteristics used to identify a standardized metric. Measurement aspect (dorsal, ventral) and side (right, left) were compared within individuals by using paired t -tests. A randomized complete block (RCB) ANOVA (SAS PROC ANOVA), with specimen as the blocking factor, was used to evaluate the effects of measurer and measurement venue (at sea versus ashore) on the mean measurement bias and precision (CVs) of pleopod measurements.

Results

Pleopod characteristics

Measurement error, and effect of side of lobster and aspect (ventral versus dorsal) on measurements Inherent measurement error averaged 0.23 mm and 0.16 mm (1.0% and 0.4%) for slipper and Hawaiian spiny lobster, respectively, based on two independent measurements by the same measurer. Exopodites of left-side pleopods averaged 3% and 2% shorter than exopodites of right-side pleopods for the two respective species (paired t -test; both $P < 0.001$; Table 1). Exopodites of first left pleopods were 4% and 2% longer in ventral aspect for slipper and spiny lobster, respectively, (RCB ANOVA; both $P < 0.001$; Table 1).

Measurement venue A matched (same-specimen) series of measurements made aboard ship versus in the laboratory (all by the same measurer) indicated a systematic difference in left pleopod length (ship > lab; RCB ANOVA; both $P = 0.001$) for slipper lobster and spiny lobster (Table 1). For each species, however, the mean difference between venues was trivial (0.2–0.4 mm or 0.6–1.4%). Differences between ship and laboratory were detectable despite the consistently lower precision provided by shipboard measurements (shipboard CVs were 47% and 39% larger for slipper and spiny lobster, respectively; RCB ANOVA: both $P < 0.001$; Table 1). Absolute differences between shipboard and lab CVs were small for the respective species (0.2% and 0.7%; Table 1).

Measurer effects An extensive series of shipboard inter-measurer comparisons between pleopod length measurements taken by one experienced (A) and a second inexperienced (B) measurer indicated trivial systematic differences between measurers (0.2%; RCB ANOVA; $P = 0.25$). Precision also was unaffected by measurer ($P = 0.31$; Table 1).

Standardized metric It follows from the above that the best measure available for use was the length (in ventral aspect) of the left first exopodite. This metric was used in all quantitative comparisons among maturity assessment methods and is recommended for future applications with these species.

Estimated sizes at functional maturity

Slipper lobster Pleopod-to-TW relations for *S. squammosus* did not differ meaningfully between 2000 and 2001 (ANCOVA; accept H_0 : slopes equal, $P = 0.11$; intercepts only 0.5% different) and both years' data were pooled for further analyses. The estimated MMP (95% CI) for the TW at which 50% of the female *S. squammosus* exhibit a disproportionately long first left exopodite was 47.6 mm (45.1–49.4 mm; Fig. 3). Estimated median body size at functional maturity based on presence or absence of berried eggs, using the same series of 2000–01 specimens, was 55.5 (52.7–58.3) mm TW (Fig. 4).

Table 1

Results of tests of potential effects of various criterion variables on the accuracy (bias of delta-bars) and precision (CVs of deltas) for measured lengths of first pleopod exopodites for slipper lobster (*Scyllarides squammosus*) and Hawaiian spiny lobster (*Panulirus marginatus*) caught from Necker Bank, Hawaii. Delta-bar = mean paired-difference; samples sizes are *n* paired observations.

Variable	Criterion	Test statistic	Delta-bar	<i>P</i>	<i>n</i>
Slipper lobster					
Body side (left vs. right)	accuracy	paired $t=-4.0$	0.9 mm	0.001	74
Measurement aspect (ventral vs. dorsal)	accuracy	RCB Anova $F_{1,62}=202.7$	0.9 mm	0.001	63
Measurement venue (shipboard vs. lab)	accuracy	RCB Anova $F_{1,62}=23.6$	0.4 mm	0.001	63
Measurement venue (shipboard vs. lab)	precision	RCB Anova $F_{1,62}=21.6$	0.7 %	0.001	63
Measurer (A vs. B)	accuracy	RCB Anova $F_{1,62}=21.3$	0.3 mm	0.001	63
Measurer (A vs. B)	precision	RCB Anova $F_{1,62}=1.54$	0.2 %	0.22	63
Spiny lobster					
Body side (left vs. right)	accuracy	paired $t=-5.7$	0.7 mm	0.001	135
Measurement aspect (ventral vs. dorsal)	accuracy	RCB Anova $F_{1,32}=31.7$	0.7 mm	0.001	33
Measurement venue (shipboard vs. lab)	accuracy	RCB Anova $F_{1,87}=11.62$	0.2 mm	0.001	88
Measurement venue (shipboard vs. lab)	precision	RCB Anova $F_{1,87}=11.74$	0.4 %	0.001	88
Measurer (A vs. B)	accuracy	RCB Anova $F_{1,87}=1.37$	<0.1 mm	0.25	88
Measurer (A vs. B)	precision	RCB Anova $F_{1,87}=1.04$	<0.2 %	0.31	88

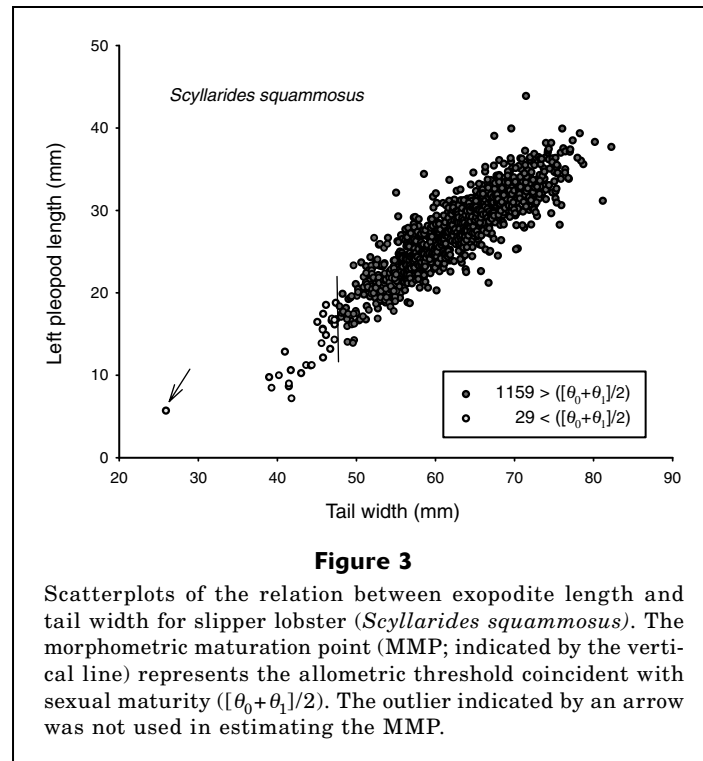
Spiny lobster Year effects on pleopod-to-TW relations for *P. marginatus* were likewise insignificant (ANCOVA; accept H_0 : slopes equal, $P>0.67$; intercepts only 0.2% different) and data for both years were pooled for further analyses. The MMP for the TW at which 50% of the *P. marginatus* females exhibit a disproportionately long pleopod was 36.4 mm (34.1–38.0 mm; Fig. 5). Figure 6 illustrates the corresponding estimate of median size at functional maturity, 35.4 (33.7–37.1) mm TW, based on the combined criteria of sperm mass and berried egg presence, for *P. marginatus*.

Estimated sizes at physiological maturity

Gonadal maturity determined from microscopic staging of histological ovary preparations indicated maturation stages ranging from oogonial to fully vitellogenic (Table 2; Minagawa and Sano, 1997) for the females of each species. For both species, gonad indices (GIs) and

median oocyte diameters generally increased over the cycle of development even though berried specimens exhibited lower GIs and oocyte sizes than unberried adults of the respective species (Table 2). The ovaries of mature females contained a preponderance of fully yolkeed oocytes whose average minimum diameter (following dehydration and staining) was 0.24 mm and 0.30 mm for *S. squammosus* and *P. marginatus*, respectively. The maximum observed diameter of fully yolkeed oocytes was 0.60 mm (in *S. squammosus*) and 0.58 mm (*P. marginatus*).

The proportions of observed immature individuals ranged from 32% to 38% of total female specimens (depending on species) and were sufficient to construct logistic curves relating percentage gonadal maturity to body size for each species. Estimated median TWs at gonadal maturity were 51.1 (48.6–53.5) mm and 40.5 (37.9–43.1) mm TW for *S. squammosus* (Fig. 4) and *P. marginatus* (Fig. 6), respectively.

**Table 2**

Stages of ovarian development in 197 slipper lobster (*Scyllarides squammosus*) and 122 Hawaiian spiny lobster (*Panulirus marginatus*) caught from Necker Bank, Hawaii. There were no stage-VI *S. squammosus*.

Ovarian stage	Characteristics of ovaries and oocytes	Gonad index mean \pm SD (range)	<i>n</i>	Most advanced oocyte substage (median diameter)
Slipper lobster	oogonia and previtellogenic oocytes conspicuous;	0.43 \pm 0.25	60	preyolk platelet
I (inactive)	ovary white	(0.02–1.08)		(0.18 mm)
II–III (developing and ripe)	unberried; developing moderately to fully vitellogenic oocytes; ovary pale orange to orange	1.63 \pm 1.45 (0.25–5.47)	75	prematuration or maturation (0.28 mm)
IV–V (ripe and redeveloping)	berried; developed fully yolked oocytes; ovary dark orange	1.12 \pm 0.66 (0.15–3.30)	62	maturation (0.26 mm)
Spiny lobster				
I (inactive)	oogonia and previtellogenic oocytes; ovary white	0.85 \pm 0.67 (0.16–2.59)	30	preyolk platelet (0.12)
II–III (developing and ripe)	unberried; developing moderately to fully vitellogenic oocytes; ovary pale orange to orange	14.03 \pm 4.46 (3.32–22.69)	42	prematuration or maturation (0.49)
IV–V (ripe and redeveloping)	berried; developed fully yolked oocytes; ovary dark orange	5.84 \pm 4.31 (0.56–17.06)	47	maturation (0.30)
VI (spent)	residual unspawned mature oocytes; ovulation traces	14.85 \pm 6.38 (7.5–18.9)	3	yolk platelet but atretic (0.48)

Discussion

Properties of the EL-TW model

In order to determine morphometric maturity, we first attempted to use a method developed by Watters and Hobday (1998). With this method splines were used to model the relationship between the morphometric character and body size; then the morphometric size at which the second derivative of the fitted curve is maximal is computed. At first this technique is alluring in that it makes no allometric or other assumption as to the shape of the relationship between the morphometric character and body size. It instead assumes that maturation corresponds to the maximum of the second derivative. This assumption is likely invalid even if we assume that the relationship between the morphometric character and body size changes abruptly at maturation for each individual (as at the pubertal molt in crustaceans) because individuals in the population mature at different sizes. When we applied the Watters and Hobday method, the resulting body size estimate appeared to characterize the minimum, not the median, size at attainment of sexual maturity in the population and was clearly inappropriate for our needs. Our method generated fitted splines that were comfortably similar in shape to the parametric logistic (sigmoidal function) models that we used to estimate maturation with berried and histological criteria.

The magnitude of the difference between the sizes at maturity estimated by our and the Watters and Hobday (1998) model should vary in proportion to the magnitude of the difference between the minimum (θ_0) and median ($[(\theta_0 + \theta_1)/2]$) body sizes at maturity and therefore be case-dependent. In our slipper lobster case, the θ_0 and θ_1 estimates differed by about 6.6 mm; hence, the two model estimates differed by about $6.6/2 = 3.3$ mm or approximately 7% of the $[(\theta_0 + \theta_1)/2]$ median. Because other cases certainly include those in which immature and adult sizes overlap even more greatly, we suggest that our more general and accurate model be adopted.

Functional versus physiological measures of maturity

Morphological features can provide adequate if imperfect measures of functional sexual maturity, as can physiological evidence for gonadal maturity (Ennis, 1984). Morphological features such as ovigerous condition can underestimate the incidence of mature individuals, but the degree to which they do so depends on numerous factors including species and population. Physiological metrics in some cases can provide more accurate estimators of both body size and age at maturity because they reveal the reproductive readiness of individuals at the time of collection. Individual body size and age at maturity can be decoupled from functional maturity metrics in Crustacea, however. For example, some crustaceans like majid crabs exhibit determinate growth following a terminal, pubertal molt (Hartnoll, 1982). For such species, size at attainment of sexual maturity is synonymous

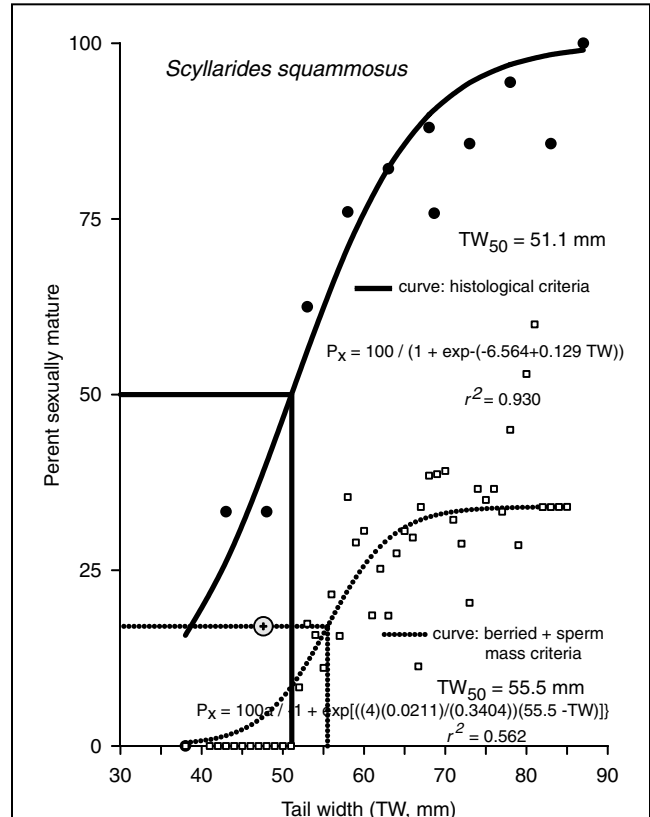
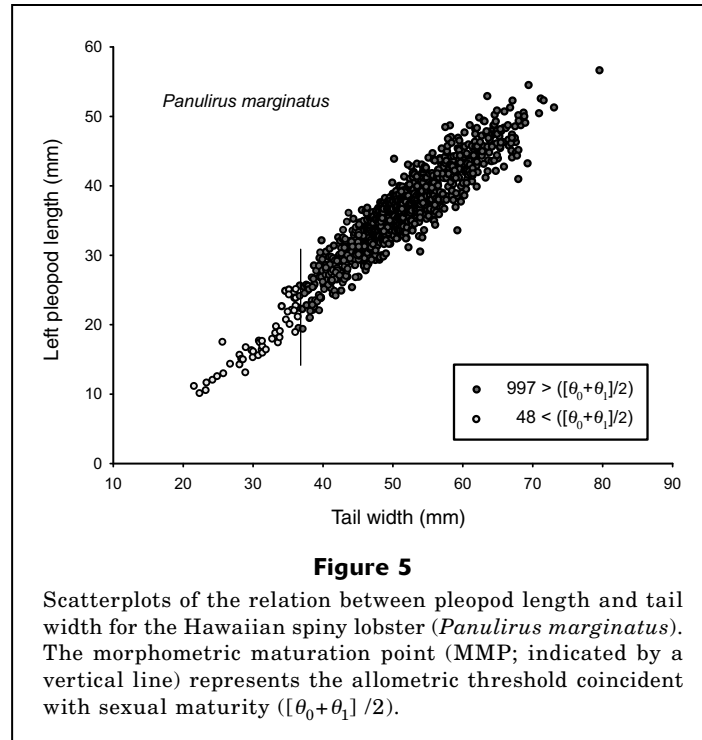


Figure 4

Scatterplots and fitted curves of the relations between body size (tail width, TW) and percent sexual maturity based on functional maturity gauged by presence-absence of berried condition (dotted curve), overlaid on gonadal maturation gauged by microscopic examination of ovaries (dark-line curve); the pleopod length-based morphometric maturation point (MMP) estimate of size at functional maturity is indicated by the large circle with cross-hairs (\oplus), for slipper lobster (*Scyllarides squammosus*). A 3-parameter logistic equation was necessary to fit the dotted curve; a 2-parameter logistic was sufficient to fit the dark-line curve (see text).

with the median body size of adults. These two attributes are not synonymous for lobsters with indeterminate growth. It is further obvious that the pleopods and other allometric body parts of Crustacea like lobsters reflect an array of gonadal maturities ranging from developing immature to fully mature, which can be problematic because some or many females might abort and resorb developing gonadal eggs after the pubertal molt (Aiken and Waddy, 1980) or may not become inseminated (Heydorn, 1969). By attributing maturity to specimens that either have not matured physiologically or that will not reproduce although capable of doing so, appendage-to-body proportions can underestimate the age at maturity in Crustacea. The degree of underestimation should be proportional to the incidence of gonadal resorption during the intermolt period following the pubertal molt,

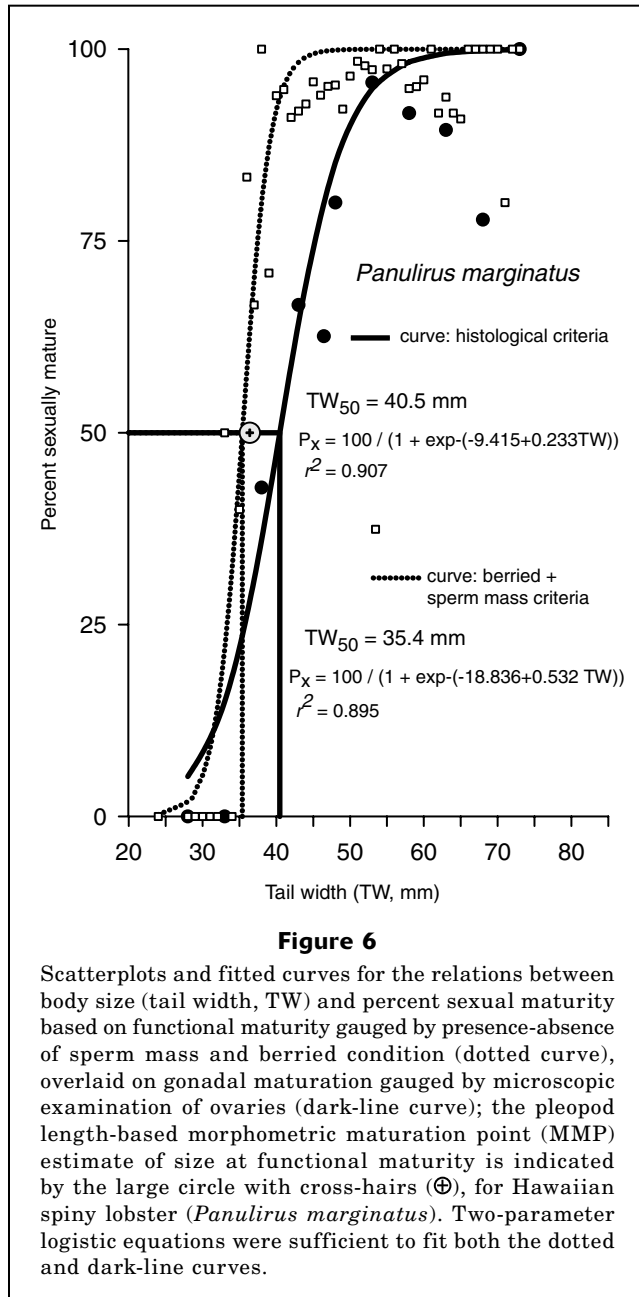


as well as the duration of the intermolt. These specific topics deserve future study.

The above caveats notwithstanding, it is helpful to compare estimates of body sizes at sexual maturity based on various morphological and physiological evidence and to ascertain the degree of agreement among the estimates (Fernandez-Vergaz et al., 2000). The estimate of MMP (47.6 mm) indicated by the pleopod length-to-TW relation for *S. squammosus*, for example, was about 16% smaller than the median size at maturity (55.5 [± 1.35 SE] mm) estimated by using simple presence-absence of berried eggs for the same series of specimens. The latter estimate, however, is imprecise and an overestimate. The long-term mean TW at 50% maturity based on berried condition for the period from 1986 to 2001, indistinguishable among component years, was 50.0 ± 0.83 mm, more precise than the single-year estimate although still biased high (DeMartini et al., 2002). If this 50.0 value is used for reference, the pleopod length-based estimate of the MMP falls within <5% of the long-term mean. For *P. marginatus*, the analogous MMP = 36.4 mm value was within 3% of the estimated median size at maturity (35.4 mm) based on the combined criteria of berried eggs and sperm mass presence. All the various estimates of functional maturity for the two species were within 2.0–12.6% (mean=7.9%) of the best respective estimate of gonadal maturity. These close similarities, despite the inherent biases of the two methods, indicate that maturity metrics such as relative pleopod length can provide highly satisfactory proxies of true functional maturity that are closely related to gonadal maturity in certain cases.

Pleopod length as a maturity metric

In some Crustacea (once again, not lobsters, as far as is known), allometries are not fixed at the pubertal molt; and, in a minority of these, allometric growth is seasonally cyclic and allometries disappear when mature instars molt during nonreproductive periods (Hartnoll, 1974, 1982). And body proportions may not be strong predictors of sexual maturity for clawed lobsters (Comeau and Savoie, 2002). In many, if not most, decapods such as spiny lobsters (e.g., George and Morgan, 1979; Groeneveld and Melville-Smith, 1994), however, relative appendage-to-body sizes, as well as obvious morphological criteria such as the presence of berried eggs and a sperm mass, indicate functional sexual maturity. Body part allometries in some cases can be better predictors of maturity than more obvious characters like berried eggs. An incomplete measure such as percentage berried, exemplified by the slipper lobster (*S. squammosus*) in the present study, can falsely fail to detect reproductively inactive adult females. Appendage-to-body size proportions thus have one major advantage over other morphometrics in that they permit reproductively inactive adult females to be correctly classified as mature. This advantage is relatively unimportant in other species like *P. marginatus* for which additional gross morphological indicators such as the presence-absence of a sperm mass complement the information provided by berried condition. Even so, proportional appendage lengths can be used in such cases as another fairly inexpensive and independent measure that could contribute to a multivariate assessment of maturity,



unconstrained by a conspicuous but perhaps inaccurate feature like berried condition.

Management implications

Estimates of body size at sexual maturity can provide key information to various stock assessment models, but only if the estimates are accurate and sufficiently precise. For the slipper lobster (*S. squammosus*), DeMartini et al. (2002) have shown that estimates made by using percent berried as the lone maturity criterion, the only morphological metric previously available, are both inaccurate and imprecise. The inability to distinguish

truly immature from mature, but reproductively inactive, females generates an inflated “immature” class, and the estimates of median size at sexual maturity thus obtained with logistic equation fits are biased high. Variances of median-size estimates based on sample sizes available on single research surveys are often so large that 3-parameter logistic applications (necessary to scale maturity to 100%) fail to converge, and reliable individual-year estimates are impossible (DeMartini et al., 2002). Unfortunately, the temporal dynamics of targeting species by fishermen in the NWHI trap fishery and the rapid phenotypic responses in fecundity and maturation size to harvesting, fluctuating natural productivity, and changing population densities that have been observed in *P. marginatus* (DeMartini et al., 2003), require that size at maturity be re-estimated at short (one-to-several-year) intervals for this species at least and possibly for *S. squammosus* as well.

The accurate and precise estimates of median body size at sexual maturity made possible by using the pleopod length metric enable such yearly re-evaluations for *S. squammosus* and provide a second reliable and independent estimator for *P. marginatus*. Our successful applications for a scyllarid as well as a palinurid, together with prior observations for numerous other spiny lobster species, indicate that easily measured appendage length-to-body size relations are generally suitable for assessing functional sexual maturity in lobsters and other decapods. We recommend that these relations be explored for other commercially exploited crustacean stocks and wherever possible routinely applied to provide cost-effective and timely information on size at maturity for stock assessments. Managers responsible for the assessment of lobster and other crustacean stocks will then have a more complete toolbox of methods generally available for assessing the size at maturity and harvestability of stocks, particularly for species like *S. squammosus* in which conventional morphological measures are inadequate.

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Literature cited

- Aiken, D. E., and S. L. Waddy.
1980. Reproductive biology. *In* The biology and management of lobsters, vol. I, physiology and behavior (J. S. Cobb and B. F. Phillips, eds.), p. 215–276. Academic Press, New York, NY.
- Berry, P. F., and A. E. F. Heydorn.
1970. A comparison of the spermatophoric masses and mechanisms of fertilization in Southern African spiny lobsters (Palinuridae). *S. Afr. Assoc. Mar. Biol. Res., Oceanogr. Res. Inst. Invest. Rep.* 25, 18 p.

- Comeau, M., and F. Savoie.
2002. Maturity and reproductive cycle of the female American lobster, *Homarus americanus*, in the southern Gulf of St. Lawrence, Canada. *J. Crust. Biol.* 22:762–774.
- Davison, A. C., and D. V. Hinkley.
1997. Bootstrap methods and their application, 582 p. Cambridge University Press, New York, NY.
- DeMartini, E. E., G. T. DiNardo, and H. A. Williams.
2003. Temporal changes in population density, fecundity and egg size of the Hawaiian spiny lobster, *Panulirus marginatus*, at Necker Bank, Northwestern Hawaiian Islands. *Fish. Bull.* 101:22–31.
- DeMartini, E. E., P. Kleiber, and G. T. DiNardo.
2002. Comprehensive (1986–2001) characterization of size at sexual maturity for Hawaiian spiny lobster (*Panulirus marginatus*) and slipper lobster (*Scyllarides squammosus*) in the Northwestern Hawaiian Islands. NOAA Tech Memo NMFS-SWFSC-344, 12 p.
- DeMartini, E. E., and H. A. Williams.
2001. Fecundity and egg size of *Scyllarides squammosus* (Decapoda: Scyllaridae) at Maro Reef, Northwestern Hawaiian Islands. *J. Crust. Biol.* 21:891–896.
- Ennis, G. P.
1984. Comparison of physiological and functional size-at-maturity relationships in two Newfoundland populations of lobsters *Homarus americanus*. *Fish. Bull.* 82:244–249.
- Evans, C. R., A. P. M. Lockwood, A. J. Evans, and E. Free.
1995. Field studies of the reproductive biology of the spiny lobster *Panulirus argus* (Latreille) and *P. guttatus* (Latreille) at Bermuda. *J. Shellfish Res.* 14:371–381.
- Fernandez-Vergaz, V., L. J. Lopez Abellan, and E. Balguerias.
2000. Morphometric, functional and sexual maturity of the deep-sea red crab *Chaceon affinis* inhabiting Canary Island waters: chronology of maturation. *Mar. Ecol. Prog. Ser.* 204:169–178.
- George, R. W., and G. R. Morgan.
1979. Linear growth stages in the rock lobster (*Panulirus versicolor*) as a method for determining size at first physical maturity. *Rap. P.-V. Reun. Cons. Int. Explor. Mer* 175:182–185.
- Gregory, D. R. Jr, and R. F. Labisky.
1981. Ovigerous setae as an indicator of reproductive maturity in the spiny lobster, *Panulirus argus* (Latreille). *Northeast Gulf Sci.* 4:109–113.
- Grey, K. A.
1979. Estimates of the size at first maturity of the western rock lobster, *Panulirus cygnus*, using secondary sexual characteristics. *Aust. J. Mar. Freshw. Res.* 30:785–791.
- Groeneveld, J. C., and R. Melville-Smith.
1994. Size at onset of sexual maturity in the south coast rock lobster, *Panulirus gilchristi* (Decapoda: Palinuridae). *S. Afr. J. Mar. Sci.* 14:219–233.
- Hartnoll, R. G.
1974. Variation in growth pattern between some secondary sexual characters in crabs (Decapoda: Brachyura). *Crustaceana* 27:131–136.
1982. Growth. In *The biology of Crustacea*, vol. I, embryology, morphology, and genetics (D. E. Bliss, ed.), p. 111–196. Academic Press, London.
- Heydorn, A. E. F.
1969. The rock lobster of the South African west coast *Jasus lalandii* (H. Milne-Edwards). 2. Population studies, behavior, reproduction, moulting, growth and migration. *S. Afr. Div. Sea Fish. Invest. Rep.* 7:1–52.
- Hogarth, P. J., and L. A. Barratt.
1996. Size distribution, maturity and fecundity of the spiny lobster *Panulirus penicillatus* (Oliver 1791) in the Red Sea. *Trop. Zool.* 9:399–408.
- Hossain, M. A.
1978. Appearance and development of sexual characters of sand lobster *Thenus orientalis* (Lund) (Decapoda: Scyllaridae) from the Bay of Bengal. *Bangladesh. J. Zool.* 6:31–42.
- Jayakody, D. S.
1989. Size at onset of sexual maturity and onset of spawning in female *Panulirus homarus* (Crustacea: Decapoda: Palinuridae) in Sri Lanka. *Mar. Ecol. Prog. Ser.* 57:83–87.
- Juinio, M. A. R.
1987. Some aspects of the reproduction of *Panulirus penicillatus* (Decapoda: Palinuridae). *Bull. Mar. Sci.* 41:242–252.
- Matthews, D. C.
1951. The origin, development, and nature of the spermatophoric mass of the spiny lobster, *Panulirus penicillatus* (Oliver). *Pac. Sci.* 5:359–371.
- Minagawa, M.
1997. Reproductive cycle and size-dependent spawning of female spiny lobsters (*Panulirus japonicus*) off Oshima Island, Tokyo, Japan. *Mar. Freshw. Res.* 48:869–874.
- Minagawa, M., and S. Higuchi.
1997. Analysis of size, gonadal maturation, and functional maturity in the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). *J. Crust. Biol.* 17:70–80.
- Minagawa, M., and M. Sano.
1997. Oogenesis and ovarian development cycle of the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). *Mar. Freshw. Res.* 48:875–887.
- Montgomery, S. S.
1992. Sizes at first maturity and at onset of breeding in female *Jasus verreauxi* (Decapoda: Palinuridae) from New South Wales waters, Australia. *Aust. J. Mar. Freshw. Res.* 43:1373–1379.
- Plaut, I.
1993. Sexual maturity, reproductive season and fecundity of the spiny lobster *Panulirus penicillatus* from the Gulf of Eilat (Aqaba), Red Sea. *Aust. J. Mar. Freshw. Res.* 44:527–535.
- Prescott, J. H.
1984. Determination of size at maturity in the Hawaiian spiny lobster *Panulirus marginatus*, from changes in relative growth. *Proc. Res. Inv. NWHI. UNIHI-SEA GRANT-MR-84-01*, p. 345. Univ. Hawaii, Honolulu, HI.
- Ratkowsky, D. A.
1983. Nonlinear regression modeling: a unified practical approach, 276 p. Marcel Dekker, New York, NY.
- Somerton, D. A.
1980. A computer technique for estimating the size of sexual maturity in crabs. *Can. J. Fish. Aquat. Sci.* 37:1488–1494.
- Watters, G., and A. J. Hobday.
1998. A new method for estimating the morphometric size at maturity of crabs. *Can. J. Fish. Aquat. Sci.* 55:704–714.

Appendix

Method for estimation of maturation with pleopod metrics

To model the allometry we used the power function $Y = \delta X^\beta$ and assumed multiplicative error. The logarithmic transformation of this function leads to a linear regression model. Specifically, we defined $\ln(Y) = f_1(X) + \varepsilon_1$, where $f_1(X) = \alpha_1 + \beta_1 \ln(X)$ and $\alpha_1 = \ln(\delta_1)$, as the allometric relationship for juvenile lobsters and $\ln(Y) = f_2(X) + \varepsilon_2$, where $f_2(X) = \alpha_2 + \beta_2 \ln(X)$ and $\alpha_2 = \ln(\delta_2)$, as the allometric relationship for adult lobsters. The errors, ε_1 and ε_2 , were assumed to be independent and normally distributed with mean 0 and variance σ_1^2 and σ_2^2 , respectively. We assumed that maturation occurred over a range of tail widths. Dividing the domain of x into four intervals, we defined the probability that a lobster with observed tail width x was mature (m) as

$$P(m|x) = \begin{cases} 0 & x < \theta_0 \\ \frac{x - \theta_0}{\theta_1 - \theta_0} \exp\left(-\gamma \left(\frac{\theta_0 + \theta_1 - 2x}{\theta_1 - \theta_0}\right)\right) & \theta_0 \leq x < \frac{\theta_0 + \theta_1}{2} \\ 1 - \frac{\theta_1 - x}{\theta_1 - \theta_0} \exp\left(-\gamma \left(\frac{2x - (\theta_0 + \theta_1)}{\theta_1 - \theta_0}\right)\right) & \frac{\theta_0 + \theta_1}{2} \leq x < \theta_1 \\ 1 & x \geq \theta_1 \end{cases} \quad (1)$$

When $\gamma = 0$, $P(m|x)$ increases linearly from 0 to 1 over the interval $[\theta_0, \theta_1]$. For $\gamma > 0$, the curves are sigmoidal, symmetrical, and the rate that the probability changes with respect to tail width is bell shaped (the sigmoidal curve first accelerates, then decelerates). The point of inflection, $(\theta_0 + \theta_1)/2$, is the tail width at which 50% of the lobsters are expected to be mature. For both species, we assumed that $\gamma \geq 0$.

Defining the allometry model and the probability of maturity as above, we expressed the model relating pleopod length to tail width as $\ln(Y) = f_1(X)(1 - P(m|x)) + f_2(X)P(m|x) + \varepsilon$, where ε are independent normal variates with mean 0 and covariance V_m .

Assuming (x_i, y_i) $i = 1, \dots, n$ independent paired observations and $\sigma^2 = \sigma_1^2 = \sigma_2^2$, $V_m = I\sigma^2 + M$, where I is the $(n \times n)$ identity matrix, M is the diagonal matrix $M_{ii} = \Delta^2(x_i)P(m_i|x_i)(1 - P(m_i|x_i))$, and $\Delta(x_i) = f_2(x_i) - f_1(x_i)$. Hence, we have a weighted least squares problem with weights

$$w_i = \begin{cases} 1 & \text{if } x_i \leq \theta_0 \text{ or } x_i \geq \theta_1 \\ \frac{\sigma^2}{(\sigma^2 + M_{ii})} & \text{if } \theta_0 < x_i < \theta_1. \end{cases} \quad (2)$$

To fit the model, we defined $\alpha_3 = \alpha_2 - \alpha_1$ and $\beta_3 = \beta_2 - \beta_1$ and expressed the model as $\ln(Y) = f_3(X)P(m|x) + f_1(X) + \varepsilon$, where $f_3(X) = \alpha_3 + \beta_3 \ln(X)$. To ensure that the curve in the transition range was monotonically increasing (if $\beta_3 > 0$), θ_0 was bounded such that $\theta_0 \geq \exp(-\alpha_3/\beta_3)$, and if $\beta_3 < 0$, θ_1 was bounded such that $\theta_1 \leq \exp(-\alpha_3/\beta_3)$. The curve was fitted by using iteratively reweighted least squares. The weights were recomputed at each iteration.

While fitting the lobster data to the specified model, we observed that one or more of the parameters involved in defining the sigmoidal curve departed from linear behavior. Under these circumstances, the confidence interval derived by assuming the asymptotic properties of maximum likelihood estimates may be invalid (Ratkowsky, 1983). Therefore, we computed approximate 95% confidence intervals for the point of inflection using the bootstrap method. Specifically, we used case resampling with 1000 bootstrap replications. Confidence intervals were derived by using the studentized bootstrap confidence limits (Davison and Hinkley, 1997).