

Use of neurolipofuscin to determine age structure and growth rates of Caribbean spiny lobster *Panulirus argus* in Florida, United States

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Abstract We histologically determined lipofuscin content in eyestalk neural tissue to estimate the age and growth of Caribbean spiny lobster (*Panulirus argus*) in Florida, United States. Neurolipofuscin was measured from size-stratified samples of 145 lobsters from the Florida Keys and 119 lobsters from the Dry Tortugas, the two main fishing areas in Florida. Modal analysis of the neurolipofuscin concentration frequency histograms suggested a relatively constant neurolipofuscin accumulation rate of 0.27% by volume (%VF) per year, which corresponded with the annual accumulation rate of 0.29%VF previously measured in the laboratory. Verification of the similarity of neurolipofuscin accumulation rates for lobsters in the field with previous laboratory studies indicates that neurolipofuscin concentration is suitable for estimating population parameters for

P. argus. Neurolipofuscin-based age estimates of lobster populations from the Florida Keys and Dry Tortugas, developed from Monte Carlo simulations of size-frequency distributions, suggested that legal-sized lobsters (>76.2 mm carapace length) in both areas were predominately 1–2 years old. Lobsters from the Florida Keys were consistently smaller for their age than those from the Dry Tortugas, suggesting that their growth is slower and that there may be little movement of lobsters between the two areas.

Keywords lipofuscin; Dry Tortugas; Florida Keys; Monte Carlo simulation; growth; Palinuridae

INTRODUCTION

The Caribbean spiny lobster, *Panulirus argus* (Latreille 1804), is one of Florida's most prized recreational fishery species and the focus of a valuable commercial fishery (Hunt 1994; Sharp et al. 2005). The management of this species is one of the most intensely contested in the state (Hunt 1994). Approximately 130 000 recreational anglers, 400 commercial divers, and 800 commercial trappers using almost 500 000 traps target spiny lobster (Florida Fish and Wildlife Conservation Commission unpubl. data). Specific management tools for the commercial fishery are a limit on the number of lobsters commercial divers can harvest each day and a trap-reduction programme intended, among other effects, to reduce the trap-induced mortality of lobsters and to develop a multiyear-class fishery (Hunt & Lyons 1986; Hunt 1994; Matthews 2001). Harvest by the recreational fishery is principally managed with daily catch limits (Sharp et al. 2005). The effect of these fishery-management programmes on lobster year classes can not be reliably evaluated because the age structure of Florida's spiny lobster population is unknown.

The Caribbean spiny lobster is widely distributed throughout the Caribbean, but lobsters in the United States are concentrated in South Florida. South

Florida is commonly considered to have two main fishing areas (Fig. 1). Near the Florida Keys is the core fishing area, used by the vast majority of commercial trap fishers, commercial divers, and recreational fishers (Hunt 1994). The Dry Tortugas are approximately 100 km west of the main islands of the Florida Keys, and the commercial and recreational fishing effort near those islands is substantially less than that near the Florida Keys (Muller et al. 1997). Most lobsters harvested near the Florida Keys are only a few millimeters above the minimum legal size of 76.2 mm carapace length (CL) (Lyons et al. 1981). Lobsters in the Dry Tortugas are generally larger and mature at a larger size (Davis 1974; Lyons et al. 1981; Bertelsen & Matthews 2001; Bertelsen et al. 2004). Although these two areas are contiguous (Fig. 1), insufficient information exists to evaluate if lobsters near the Florida Keys and Dry Tortugas are two distinct populations or to adequately explain why lobsters in these two areas have different size-frequency distributions and sizes at maturity.

Estimating the age of crustaceans by measuring size (CL or weight) is highly inaccurate. Generally, a positive relationship exists between size and age, but regression analyses show that this relationship is almost always statistically insignificant in crustaceans (Sheehy 1990a; Sheehy et al. 1996, 1998; Wahle et al. 1996; Bluhm & Brey 2001; Bluhm et al. 2001; Sheehy & Bannister 2002; Uglem et al. 2005). Age and growth of *P. argus* in Florida have been estimated principally by tag-recapture studies (Davis 1978; Lyons et al. 1981; Hunt & Lyons 1986; Davis & Dodrill 1989; Forcucci et al. 1994; Sharp et al. 2000). These studies seldom include observations of individual lobsters for longer than one year or larger than 100 mm CL. Low tag-recapture rates or short periods at large are probably related to the high mobility of the species (Herrnkind 1980) and high fishing pressure (Muller et al. 1997). Tag-recapture methods may also introduce tag retention and lobster mortality issues. A tag-retention study of sphyron-anchor spaghetti tags (Hallprint Pty Ltd, Victor Harbor, South Australia) indicated that more than 75% of tagged lobsters either lost their tags or died within 23 weeks of tagging, and surviving lobsters that were tagged moulted less frequently than control lobsters (Florida Fish and Wildlife Conservation Commission unpubl. data). Consequently, more tag returns are probably from lobsters that moult less frequently, and the resulting analyses probably underestimate growth.

The use of age pigments, called lipofuscin, has proven to be a more reliable determinant

of age of animals, including crustaceans, than animal size (Sheehy 2002). Lipofuscin consists of autofluorescent-lysosomotropic compounds that accumulate in tissues as animals age (Eldred & Lasky 1993). They likely accumulate as a function of physiological age rather than strict chronological age (Sohal & Donato 1979; Katz et al. 1984; Sheehy 1990b; Sheehy et al. 1995). However, the regression of lipofuscin concentration versus chronological age is generally highly significant, unlike size versus chronological age. The relationship between lipofuscin concentration and age has been demonstrated in European lobster, *Homarus gammarus* (Sheehy et al. 1996, 1999) and Western Australian rock lobster, *P. cygnus* (Sheehy et al. 1998). Neurolipofuscin accumulated with age in *P. argus* raised in the laboratory under natural temperature conditions, and although accumulation oscillated seasonally with temperature, there was an overall linear trend between years (Maxwell et al. 2007).

Quantifying the lipofuscin concentration by histologically examining tissue, although time consuming, has proven to be the most reliable and precise method available (Sheehy 1990a). The central nervous tissue, especially olfactory regions of the brain and portions of the eyestalk, are the tissue of choice for measuring lipofuscin histologically (Sandeman et al. 1992). In particular, neurites of the cell bodies associated with the olfactory lobes in the brain and within the terminal medulla in the eyestalks are the preferred tissue analysed because of their relatively high and age-correlated concentration of lipofuscin (Sheehy et al. 1996). Eyestalk tissue is also relatively easy to collect.

In this study, we attempted to verify the rate of neurolipofuscin accumulation in wild lobsters and thus evaluate if neurolipofuscin concentration is a suitable indicator of age for Caribbean spiny lobster in Florida. A strong relationship between age and neurolipofuscin concentration was described using known-age lobsters in the laboratory (Maxwell et al. 2007), but the relationship requires verification in wild lobsters. Herein, we developed neurolipofuscin concentration-frequency histograms and estimated the growth of lobsters from the two main fishing areas in Florida: the Florida Keys and Dry Tortugas. Age and size are the most basic parameters required to understand growth, maturation, longevity, and mortality. Robust estimates of these parameters will improve stock assessments and improve our understanding of the connection between the lobster populations at the Florida Keys and Dry Tortugas.

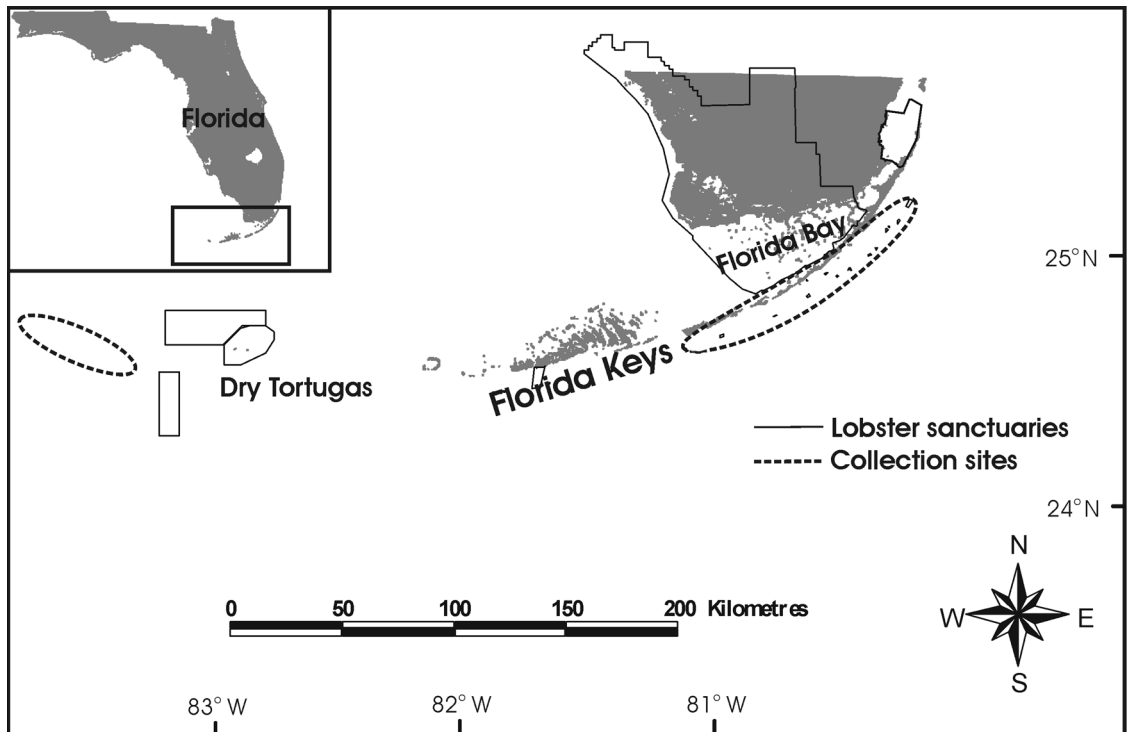


Fig. 1 Map of South Florida, United States showing study sites at the Florida Keys and Dry Tortugas.

MATERIALS AND METHODS

Sampling and study area

We measured neurolipofuscin concentrations in wild-caught lobsters from two length-stratified samples, one collected near the Florida Keys and one near the Dry Tortugas (Fig. 1). Lobsters were collected from commercial lobster traps. We used a CL-stratified sampling technique to ensure capture of a wide size (and presumably age) range of lobsters. At the Florida Keys, we collected 145 lobsters, approximately 10 male and 10 female lobsters from each of eight 10-mm size categories between 40 and 120 mm CL during January and February 2001. Fewer female lobsters were available in the 40- ($n = 4$), 100- ($n = 4$), and 110-mm CL size classes ($n = 3$). We collected 119 lobsters at the Dry Tortugas during January 2002. Approximately 5 male and female lobsters were collected in each of ten 10-mm CL size categories between 80 and 170 mm CL. No lobsters smaller than 80 mm CL and only 5 female lobsters larger than 150 mm CL were collected.

Size-frequency distributions of male and female lobsters were created from fishery-observer data for

lobsters caught in traps at the Florida Keys and from port-sampler data for lobsters caught in traps at the Dry Tortugas. Fishery observers at sea measured 2413 male and 3160 female lobsters, including both undersized and legal-sized lobsters caught at the Florida Keys during January and February 2001. Port samplers measured 304 male and 393 female legal-sized lobsters caught at the Dry Tortugas during January–March 2002.

Lipofuscin identification and measurement

Neurolipofuscin was quantified based on Maxwell et al. (2007) as modified from Sheehy et al. (1996). We histologically determined lipofuscin concentration in eyestalk neural tissue. Eyestalks were removed from anaesthetised animals, immediately placed into 4% paraformaldehyde for 48 h, and transferred into 0.2 mol litre⁻¹ phosphate buffer plus azide. The internal neural tissue of the eyestalk was dissected from the cuticle, dehydrated, and embedded in paraffin. Eyestalk tissue was sectioned at 6 μ m thickness from the proximal end of the nerve tract connecting the hemiellipsoid body to cluster A and continuing distally through the nerve tract. Sections were excited

with blue light at 450–490 nm and emission was detected at 515–585 nm by using a Zeiss Axioplan fluorescence microscope with a 100× oil-immersion objective. In each section, the area of cluster A that contained the highest neurolipofuscin concentration was imaged. Depending on the size of the animal, 15–40 (usually 25–30) images were captured per eyestalk by using a Carl Zeiss ZVS-3C75DE camera fitted to the microscope. Images were acquired using Digital Camera Acquire (Interactive Acquisition Utility, v.3.0) software and saved as 800- × 600-resolution bitmap files. Images were analysed using Adobe Photoshop 6.0. Colour images were converted to greyscale, and for each tissue section, a region of interest was selected and the cross-sectional areas of neurolipofuscin and background tissue within it were quantified. We used a weighted geometric average of the area fractions of neurolipofuscin in all images as a measure of the neurolipofuscin concentration in the individual (Sheehy et al. 1998). Following stereological convention, this average area fraction is reported as a percentage volume fraction (%VF).

We used neurolipofuscin values determined from one eyestalk for each lobster from the Florida Keys and the average value from both eyestalks for each lobster from the Dry Tortugas. Only one eye was successfully processed for 13 of the lobsters from the Dry Tortugas. We used a paired *t* test to evaluate inter-eye variability in lipofuscin values of lobsters from the Dry Tortugas (Sokal & Rohlf 1987, $\alpha < 0.05$). Age was estimated for animals collected at the Florida Keys and Dry Tortugas by applying the linear regression describing neurolipofuscin accumulation in known-age animals raised in the laboratory (Maxwell et al. 2007). There was no difference in neurolipofuscin accumulation rates between males and females of known age, so the regression equation from Maxwell et al. (2007) was

applied to both sexes. The linear regression equation of neurolipofuscin concentration on age is:

$$N = 0.290t - 0.119$$

where *t* = time (years) and *N* = percentage volume fraction (%VF) of neurolipofuscin ($r^2 = 0.61$).

Verification of neurolipofuscin accumulation rate and age distributions

Neurolipofuscin concentration-frequency histograms were based on the CL-stratified samples collected from the Dry Tortugas and Florida Keys. A class interval width of 0.05%VF was chosen to allow for 5 to 6 class intervals per year, to facilitate interpretation of subsequent modal analyses. Potential cohorts in the histograms were fitted to models using ELEFAN and Bhattacharya's method (Gayanilo & Pauly 1997; Gayanilo et al. 2002). In all instances, modal separation indices were above the critical value of 2.0 as reported subsequently.

Standard age-frequency histograms were constructed from neurolipofuscin-based age-estimates of lobsters from the Florida Keys and Dry Tortugas by using 100 runs of a Monte Carlo simulation that randomly assigned neurolipofuscin values from each legal-sized lobster (≥ 76.2 mm CL) in the size-stratified samples to an individual lobster of the same CL (± 1 mm) in the size-frequency distributions. The Monte Carlo approach addresses the likely bias introduced by selectively sampling the entire size range, which is required to include rare large lobsters in the population. The resulting simulated neurolipofuscin-concentration histograms for the Florida Keys ($n = 843$) and Dry Tortugas ($n = 697$) were examined for potential cohorts and intermodal distance. Potential cohorts for the mean frequency of the 100 simulations for each neurolipofuscin interval in the histograms were fitted to models using ELEFAN and Bhattacharya's method as described

Table 1 Results of modal analysis of neurolipofuscin concentration-frequency histograms from a carapace-length stratified sample of lobsters from the Florida Keys ($n = 145$) and Dry Tortugas ($n = 119$), United States.

Area	Group designation	Mean	SD	Separation index
Dry Tortugas	age 1	0.264	0.068	–
	age 2	0.508	0.104	2.84
	age 3	0.800	0.065	3.46
	age 4	1.086	0.067	4.33
Florida Keys	age 1	0.263	0.100	–
	age 2	0.575	0.045	3.82
	age 3	0.797	0.810	2.86

previously (Gayanilo & Pauly 1997; Gayanilo et al. 2002). Age-0 was defined as the first post-puerulus stage.

Growth parameters

The relationships between neurolipofuscin-estimated age and CL in male and female lobsters from the Florida Keys and Dry Tortugas were fitted with von Bertalanffy growth curves (Gayanilo et al. 2002) using ELEFAN:

$$CL_t = CL_\infty (1 - e^{-k(t-t_0)})$$

where CL_t = carapace length (mm) at age t (years), CL_∞ = theoretical mean carapace length of the oldest lobsters, k is a curvature parameter, and t_0 = theoretical age at pueruli metamorphosis, $CL = 6$ mm (Gayanilo & Pauly 1997; Gayanilo et al. 2002). Age for individual lobsters was assigned based on the linear relationship between neurolipofuscin concentration and known age developed for lobsters raised in the laboratory (Maxwell et al. 2007). CL_∞ was set at 179 mm CL for males and 155 mm CL for females based on the upper 99th% distribution of male or female lobsters from both the Florida Keys and Dry Tortugas combined.

RESULTS

Lipofuscin identification and measurement

Lipofuscin concentrations of replicate left and right eye samples from Dry Tortugas lobsters were not different ($t = 2.29$; d.f. = 105; $P > 0.05$). However, 5–10% of replicate neurolipofuscin samples from eyestalk neural tissue had high levels of variability and three paired samples differed by between 1 and 2 SD. We observed that some eyes with visible injuries had neurolipofuscin concentrations that differed from expected values, but these injuries were not present in all eyes that had unexpected neurolipofuscin quantities and some injured eyes had expected values. Subsequent visual examinations of lobster eyes from wild populations indicated that about 3% of lobsters had eye injuries. Neurolipofuscin granule size in wild lobsters, although not specifically measured, tended to increase with lobster size, and granules tended to be concentrated near the periphery of neural somata cluster.

Verification of neurolipofuscin accumulation rate and age distributions

Modal analysis of the neurolipofuscin concentration-frequency histograms for lobsters collected in the

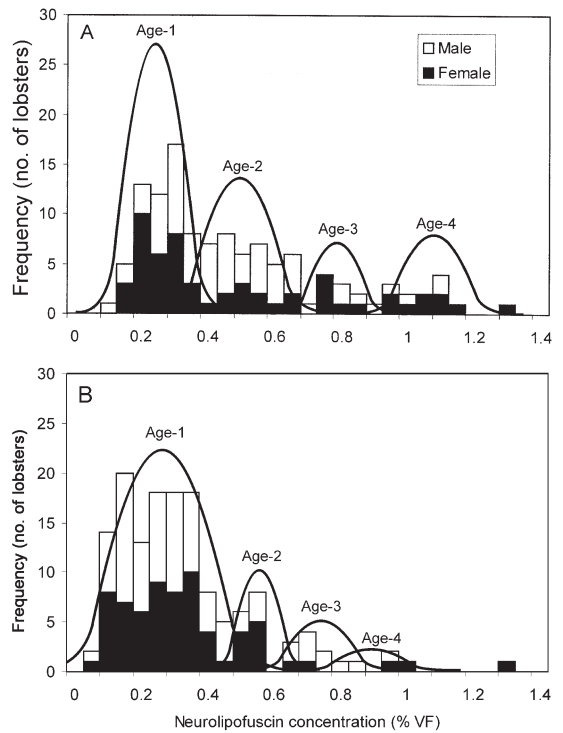


Fig. 2 Neurolipofuscin concentration-frequency histograms of carapace-length stratified samples for lobsters from **A**, the Dry Tortugas ($n = 63$ for males and 56 for females) and **B**, the Florida Keys ($n = 77$ for males and 68 for females). Also shown are sex composition of modes, fitted normal components, and annual cohort designations.

size-stratified sample of lobsters from the Florida Keys and Dry Tortugas revealed an average neurolipofuscin accumulation rate of 0.27% VF per year. The initial neurolipofuscin mode for lobsters from the Dry Tortugas was identified at 0.264, and subsequent modes had accumulation rates of 0.244, 0.292, and 0.286 (Table 1). All separation indices were above 2.0, although age-3 and age-4 cohorts were not visually distinct (Fig. 2A). The oldest age estimated for lobsters in this sample was age-5 (Fig. 2A). Modal analysis of the neurolipofuscin concentration-frequency histograms of lobsters collected in the size-stratified sample of lobsters at the Florida Keys suggested the presence of three modes and a few predominately female lobsters over age-3 in the sample. The initial neurolipofuscin mode was identified at 0.263 and the subsequent modes were separated by 0.312 and 0.222 (Table 1, Fig. 2B).

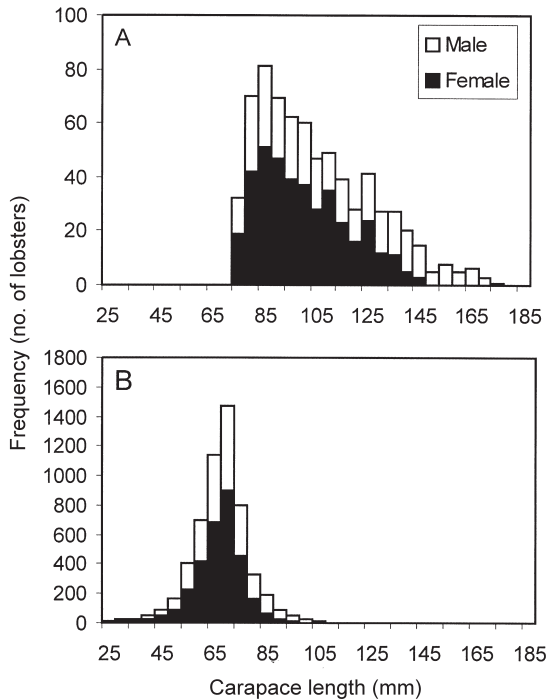


Fig. 3 Length-frequency distributions for male and female lobsters captured in traps from **A**, the Dry Tortugas ($n = 304$ for males and 393 for females) and **B**, the Florida Keys ($n = 2413$ for males and 3160 for females).

Size-frequency distributions of male and female lobsters from both the Florida Keys and Dry Tortugas showed no indication of cohorts (Fig. 3A,B). In both size-frequency distributions, legal-sized lobsters (>76.2 mm CL) steadily decreased in abundance with increasing size. The frequency of lobsters <65 mm CL reflects trap artifacts not relative lobster abundance (Fig. 3B).

Neurolipofuscin-based age estimates of lobsters, developed from Monte Carlo simulations of size-frequency distributions, suggested that most lobsters at both the Florida Keys and Dry Tortugas were predominately 1–2 years old (Fig. 4). Age-1 cohorts at both the Florida Keys and Dry Tortugas included 84% and 67% of the population in each area, respectively. The age-2 cohort included approximately 16% of the lobsters at the Florida Keys and 22% of the lobsters at the Dry Tortugas. Less than 1% of the lobsters at the Florida Keys were age-3 or older. At the Dry Tortugas, 7% of the lobsters were age-3 and 4% were age-4. A few,

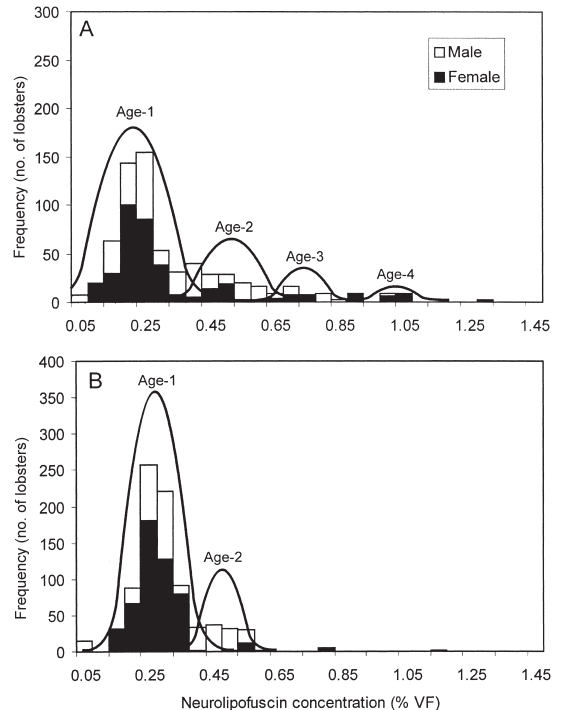


Fig. 4 Monte Carlo simulated neurolipofuscin-frequency distribution for male and female lobsters captured in traps from **A**, the Dry Tortugas ($n = 63$ for males and 56 for females) and **B**, the Florida Keys ($n = 39$ for males and 33 for females). Also shown are sex composition of modes, fitted normal components, and annual cohort designations.

mostly female lobsters were older than age-4 (Table 2, Fig. 4). Monte Carlo simulated age-frequency histograms of lobsters from both the Florida Keys and Dry Tortugas included age estimates for some legal-sized lobsters that were below neurolipofuscin concentrations of 0.15 %VF corresponding to less than 6 months of age (Fig. 4).

Growth parameters

The von Bertalanffy equations were calculated for male and female *P. argus* at the Florida Keys and Dry Tortugas (Table 3). The von Bertalanffy models visually fit age-size data for male lobsters from the Dry Tortugas ($n = 63$, $r^2 = 0.56$). Female lobster from the Dry Tortugas and both male and female lobsters from the Florida Keys were considerably smaller than the CL_{∞} constrained models indicated, and the model did not fit these three groups as well ($n = 56, 77, 68$; $r^2 = 0.26, 0.32, 0.16$, respectively). An alternative von Bertalanffy growth model, not constrained by realistic values of CL_{∞} , fit the data

better ($r^2 = 0.56, 0.45, 0.45,$ and 0.41 for males and females from the Dry Tortugas and males and females from the Florida Keys, respectively). However, the calculated CL_∞ for the Florida Keys for both male ($CL_\infty = 115$ mm) and female ($CL_\infty = 101$ mm) lobsters were unrealistically small given the observed size of lobsters at the Dry Tortugas. All lobsters over 2.2 years old in our samples from both the Florida Keys and Dry Tortugas had much slower growth rates than the younger lobsters (Fig. 5).

The linear relationship between CL and neuro-lipofuscin concentration in male and female lobsters at the Florida Keys ($n = 145, r^2 = 0.36$) was more variable than it was in male and female lobsters at the Dry Tortugas ($n = 119, r^2 = 0.46$). The relatively poor

fit of von Bertalanffy growth models for lobsters from the Florida Keys was related to the high variability in size at age. Lobsters at the Florida Keys that were estimated to be 16 months old, based on an observed neurolipofuscin concentration of 0.49%VF had CLs ranging from 40 to 110 mm. At the Dry Tortugas, 16-month-old lobsters had less variable CLs ranging from 80 to 120 mm (Fig. 5). We could not determine if the Dry Tortugas population also included slow-growing individuals less than 1 year old because of a lack of individuals under 80 mm CL. The absence of lobsters over 110 mm CL at the Florida Keys greatly affected the calculation of CL_∞ and impaired calculation of growth rates ($k > 0.73$ for all models) when CL_∞ was not constrained.

Table 2 Results of modal analysis of neurolipofuscin concentration-frequency histograms from a Monte Carlo simulation of the legal-sized lobster in the Florida Keys ($n = 72$) and Dry Tortugas ($n = 119$), United States.

Area	Group designation	Mean	SD	Population	Separation index
Dry Tortugas	age 1	0.217	0.103	67%	—
	age 2	0.536	0.082	22%	3.45
	age 3	0.762	0.052	7%	3.37
	age 4	1.025	0.059	4%	4.74
Florida Keys	age 1	0.269	0.060	84%	—
	age 2	0.479	0.045	16%	4.08

Table 3 Von Bertalanffy parameters (CL_∞ , theoretical mean carapace length of oldest 99th percentile; k , curvature parameter) for male and female lobsters from the Florida Keys and Dry Tortugas, United States. CL_∞ was set at the upper 99% size distribution for all lobsters observed in South Florida. Laboratory parameters from Matthews & Maxwell (2007).

Sex and location	Parameter	Value	r^2	SE of estimate	CV of estimate
Florida Keys					
Males	CL_∞	179	0.32	56.7	0.32
	k	0.40		0.25	0.63
Females	CL_∞	155	0.16	31.71	0.20
	k	0.45		0.21	0.48
Dry Tortugas					
Males	CL_∞	179	0.56	17.57	0.1
	k	0.66		0.25	0.38
Females	CL_∞	155	0.26	8.7	0.06
	k	0.79		0.20	0.25
Laboratory					
Males	CL_∞	180			
	k	0.49			
Females	CL_∞	145			
	k	0.61			

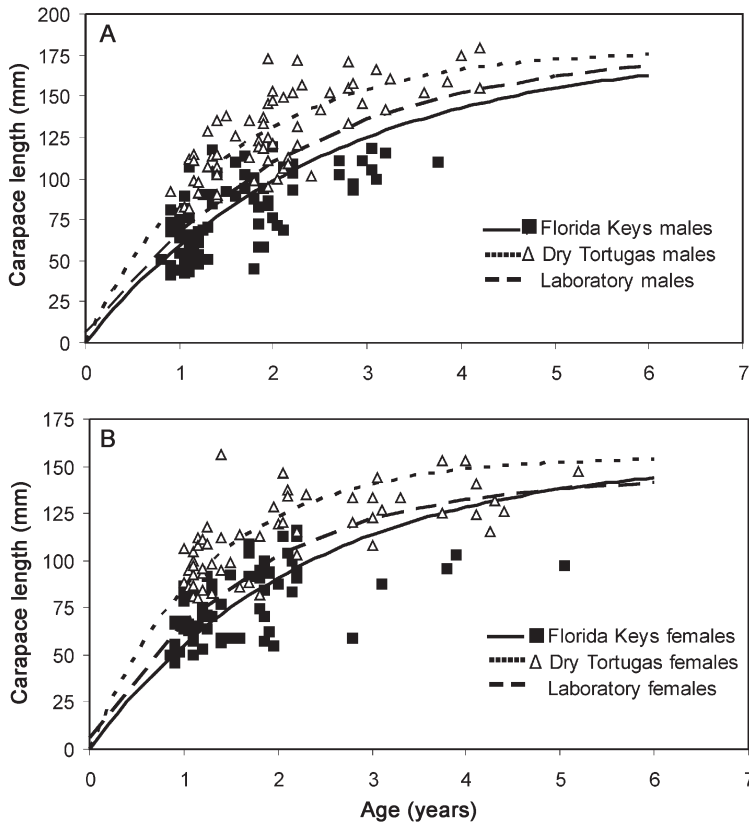


Fig. 5 Carapace length-age distribution for **A**, male and **B**, female lobsters at the Florida Keys and Dry Tortugas. Von Bertalanffy growth curves set CL_{∞} (theoretical mean carapace length) based on the upper 99% size distribution for all lobsters observed in South Florida. Reference von Bertalanffy growth curves for lobsters raised in the laboratory from Matthews & Maxwell (2007).

DISCUSSION

The verification of predictable neurolipofuscin accumulation rates in wild lobsters is a critical step in the use of this ageing technique to develop age estimates and determine population parameters (Sheehy et al. 1998; Kodama et al. 2006). Modal progression in neurolipofuscin-concentration histograms of lobsters at the Dry Tortugas provided confirmation that this technique is suitable for measuring the age of Caribbean spiny lobster. The relatively constant neurolipofuscin accumulation rate of 0.27%VF for lobsters from the Dry Tortugas corresponded with the annual accumulation rate of 0.29%VF previously measured in the laboratory (Maxwell et al. 2007). There was considerable consistency between cohort mode locations for lobsters from the Florida Keys and Dry Tortugas. Overall, neurolipofuscin concentration is a suitable proxy for age and may be used to estimate population parameters for *P. argus*. However, the age of some individual lobsters could not be

consistently determined and a few lobsters appeared conspicuously young for their size.

The variability between the concentrations of neurolipofuscin observed in replicate samples identified a likely source of error that limits sampling precision when using a single sample. The level of variability between replicate samples was consistent with the level of variability in laboratory experiments on known-age lobsters (Maxwell et al. 2007). Potential causes for poor replication were identified in *Penaes japonicus*, where neurolipofuscin granule size was different at various ages and had an irregular pattern that was not consistent with age; nonetheless, granule density and area fraction were age-related (Vila et al. 2000). Eyestalk ablation has been shown to alter the neurolipofuscin accumulation in the remaining eye (Fonseca et al. 2003), and it may have been possible that internal or physiological abnormalities in the eyes accounted for the abnormal neurolipofuscin estimates. Although not explicitly tested, there may be a link between injury to the eye and neurolipofuscin accumulation. Regardless of the

cause of the anomalous neurolipofuscin estimates, few replicate samples deviated significantly, and as suggested by Maxwell et al. (2007), the effort to process replicate samples and identify the erroneous samples might be better spent increasing the sample size.

Measurements of neurolipofuscin concentration were successfully used to create simulated age-frequency distributions of lobsters from the Florida Keys and Dry Tortugas to identify annual cohorts. Previous research using size-based age estimates (Muller et al. 1997) and the size-frequency distributions in this study suggested a lack of cohorts for lobsters in Florida. Additionally, size-frequency distributions of trap-caught lobsters at the Florida Keys varied little between years and had a single mode near the legal-size limit of 76.2 mm CL (Lyons et al. 1981; Hunt & Lyons 1986; Bertelsen et al. 2004). The year-round, albeit seasonal, recruitment pattern of pueruli to Florida may also limit the development of cohorts (Acosta et al. 1997). One factor which might have allowed the identification of age cohorts for this species in Florida may have been the subtropical climate. Lobsters in Florida are subject to greater seasonal fluctuations in temperature than most other Caribbean spiny lobster populations, and winter temperatures in Florida Bay are not conducive to lobster growth (Field & Butler 1994) or neurolipofuscin deposition (Maxwell et al. 2007). Neurolipofuscin use as an indicator of physiological age instead of chronological age would only minimally affect our estimate of the contribution of each annual cohort to the total lobster population. Those pueruli settling in winter might have slower neurolipofuscin accumulation and could potentially have similar neurolipofuscin concentrations as pueruli settling in spring, thus causing a distinct separation between annual cohorts. The limited number of annual cohorts in both the Florida Keys (2) and Dry Tortugas (4) was unexpected given the estimated 20-year longevity of spiny lobsters (Kanciruk 1980, Maxwell et al. 2007) especially at the Dry Tortugas, which has been commonly considered an area with multiple age-classes of lobsters and lower fishing pressure than the Florida Keys (Muller et al. 1997).

The von Bertalanffy growth equations developed in this study indicated substantially higher growth rates for lobsters in the first 2 years than those reported in other studies (FAO 1998; Wahle & Fogarty 2006). The lack of old lobsters that have grown to a large size at the Florida Keys and Dry Tortugas caused the calculation of a low CL_{∞} for

this species. There appeared to be rapid growth of lobsters from the Florida Keys and Dry Tortugas for approximately 2 years and then growth asymptoted. This growth pattern is consistent with the observed size distributions of both male and female lobsters at both locations (Bertelsen et al. 2004) and the paucity of lobsters over 120 mm CL at the Florida Keys. CL_{∞} varies throughout the Caribbean from 169 mm CL for males and 139 mm CL for females to 257 mm for males and 209 mm CL for females (FAO 1998). Our determination of CL_{∞} for lobsters from the Dry Tortugas was consistent with the lower estimates of CL_{∞} from the region, but our estimates for CL_{∞} at the Florida Keys were considerably lower. Our estimates of k were substantially higher than most estimates (FAO 1998), although our estimates of growth were within the upper range for *P. argus* in Cuba (Phillips et al. 1992). The high SEs for k for Florida Keys lobsters were likely caused by the high variability in size for age-1 and age-2 lobsters. The relatively low asymptote estimated by von Bertalanffy growth equations was likely an artifact of the high removal rate of lobsters by the fishery. High fishing pressure likely caused few lobsters to exceed 3 years age or be larger than 110 mm CL. The few older yet small Florida Keys lobsters appeared to have suppressed growth rates and they were likely below the minimum legal size for harvest and unavailable to the fishery for much of their lives.

Determination of *P. argus* growth rates using neurolipofuscin-based age estimates eliminates the biases associated with tag-recapture methods, which may be one of the reasons that estimates of growth in this study were near the maximum rates reported in other studies. However, high growth rates were also found in laboratory studies where the average size of lobsters reached 63 mm CL at 1 year of age for both sexes and 100 and 109 mm CL for female and male lobsters, respectively, at 2 years (Matthews & Maxwell 2007). Lobster growth in Florida Bay was estimated at 42 mm CL per year based on a study that used minimally invasive microwire tags (Sharp et al. 2000). The same microwire-tag study also reported some individuals growing to 55 mm CL in less than 1 year and some individuals that were recaptured before 1 year had projected growth rates to 75 mm CL in 1 year. Phillips et al. (1992) also reported individual *P. argus* with projected growth rates of over 75 mm CL at 1 year. Growth rates estimated from recaptured lobsters tagged with the more invasive spaghetti tag (Hallprint™) generally included many lobsters that did not moult (Warner et al. 1977; Davis 1981; Lyons et al. 1981; Hunt

& Lyons 1986; Forcucci et al. 1994; Muller et al. 1997). Lack of observable growth is an inherent problem in the studies of crustaceans and particularly for lobsters in Florida because incremental growth may not be observed during the short recapture intervals.

Differences in growth rates between the Florida Keys and Dry Tortugas lobsters may be the result of several factors. Higher growth rates of Dry Tortugas lobsters could be caused by differential survival of larger and presumably faster-growing individuals in this area. A higher predation rate on lobsters at the Dry Tortugas is consistent with the high relative abundance of predators in that area as compared with the heavily fished Florida Keys (Ault et al. 1998, 2002). Additionally, lower growth rates at the Florida Keys may be the result of fishery practices that injure or starve undersized lobsters while they are confined in traps. Commercial trap fishers use undersized lobsters as live bait (Heatwole et al. 1988), often confining up to 1.5 million lobsters each day (Hunt et al. 1986; Matthews 2001). Injuries are known to reduce the growth rate of lobsters in Florida (Davis 1981; Hunt et al. 1986). The recreational fishery for lobsters may be the most intensive in the world (Sharp et al. 2005) and hundreds of thousands of undersized lobsters may be injured when handled by fishers (Parsons & Eggleston 2005). The combined effect of these fishing practices appears to have sufficient potential to reduce growth rates for undersized lobsters in the Florida Keys.

Our estimates of growth and age structure for the Florida Keys and Dry Tortugas lobsters were markedly different, suggesting that lobsters in these areas are somewhat isolated from each other. It is commonly believed that Florida Bay and the nearshore waters of the Florida Keys are the nursery area for many lobsters in South Florida, these lobsters move to nearby reefs as they mature and some lobsters potentially continue moving to Florida's east coast or the Dry Tortugas (Gregory & Labisky 1986). The lack of small lobsters and the abundance of large lobsters at the Dry Tortugas were also observed by fishery-independent studies that included observations of undersized lobsters (Bertelsen & Cox 2001; Bertelsen & Matthews 2001) and support the hypothesis that Dry Tortugas lobsters are immigrating as adults from elsewhere. However, our estimates of the age structure and growth rates of the Dry Tortugas lobsters are inconsistent with the Florida Keys being the source of that immigration. A second hypothesis is that sufficient numbers of pueruli recruit to the Dry Tortugas and survive to

maintain the population. Extensive surveys of lobsters at the Dry Tortugas suggested that juvenile lobsters were restricted to a few small areas and only occur periodically (Bertelsen & Cox 2001; Bertelsen et al. 2004) and that there may not be adequate numbers of juvenile lobsters to maintain the adult population. The remaining hypothesis is that lobsters at the Dry Tortugas may have immigrated from elsewhere. The lobster population north of the Florida Keys, in the Gulf of Mexico, is poorly understood, but appears to be the only remaining alternative. These Gulf of Mexico lobsters are often characterised as large, often 0.75 kg total weight, non-reproductive individuals, and one tag-recapture study identified a strong westward movement pattern for these lobsters towards the Dry Tortugas (Gregory & Labisky 1986). If these lobsters are non-reproductive because they are immature, they may represent a young, fast-growing population, which is consistent with this study's observation of growth rates for lobsters at the Dry Tortugas. Additional research is required to establish if there is a link between lobsters in the Gulf of Mexico and at the Dry Tortugas.

Neurolipofuscin concentration appears suitable for estimating population parameters for *P. argus*. Our initial application of this information to the fishery in South Florida suggests that both the Florida Keys and Dry Tortugas fisheries predominantly comprise two year-classes for the majority of landings, that the two areas of the fishery may be more independent of each other than previously hypothesised, and that fishing practices may affect the growth of lobsters at a much broader scale than previously considered. Methods for the detection of neurolipofuscin need to be made more cost effective so that they can be applied more broadly in invertebrate research and more consistently between years for stock assessment studies. Specifically, for the Caribbean spiny lobster, additional research is needed to verify the growth rates identified in this study.

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