Year 3 Progress Report and a request for Supplemental Funds for year 4.

"Aspects of the ecology of the red squid *Ommastrephes bartramii*, a potential target for a major Hawaiian fishery"

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Introduction

The red squid, *Ommastrephes bartramii*, is one of the dominant large animals in the North Pacific ecosystem as indicated by its historical fishery yield (Fig. A). Our project is focused on this squid during its southern spawning migration and the portion of the population that we encounter (other than paralarvae) is virtually restricted to mature males and females (Fig. B). Our ability to study this squid depends on a two-week cruise to collect data in February of each year on the FTS HOKUSEI MARU from the University of Hokkaido, Japan. The cruise plan for February 1999 was to run west to 164° W then take a series of stations beginning well south of the Hawaiian Archipelago and passing through the Archipelago and further north through the subtropical front. This was essentially the same as the 1998 plan, which was altered due to bad weather conditions. Weather conditions also caused some alterations to 1999's cruise plan (Fig. 1).

This past February, the subtropical front was unusually far north and especially well defined. At one station, directly on the front, we experienced a two-degree C drop in temperature during the night as we were slowly pushed across the front by the wind. As in past years, the appearance of the zooplankton at the front dramatically changes with tows being dominated by the subtemperate copepod *Calanus pacificus*. Our attempt to sample north of the front was limited by bad weather, which drove us south-eastward. The next station allowed by the weather was about two degrees of latitude further south but, surprisingly, still near the front, which had also curved strongly to the south (Fig. 1). We then proceeded to Nihoa to get stations to the immediate north and south of the Archipelago.

Fishing gear

We continue to experiment with fishing methods. We use drift-jiglines to compliment sampling with poles and hand lines and with automatic jigging machines. At the front, squid caught on the drift-jiglines showed unusually high levels of cannnibalism after the typical 3-4 h of "soaking time." Presumably this was due to the high abundance of squid in the region. At the southern stations where we suspect that female red squid are present but not in surface waters, we tried deep hand-line fishing. This resulted in the capture of a single female at about a depth of 100m.

Distribution of adult male and female red squid

As was typical of previous cruises, the catch of males was much greater than the catch of females. A total of 79 males and 25 females were taken (Fig. 2). The size of the males was comparable to that of 1996 and 1998 in contrast to the distinctly larger size of males during 1997 (Fig. 3). The females were also large in 1997, however, the 1999 females strongly overlapped the 1997 females in size (their median difference is not significant, p = 0.2) (Fig. 4). The single female captured on the deep hand-line to the south was unusually small (446 mm ML) yet heavy for its size even though the oviducts were virtually empty. We are anxious to learn if the tissue samples taken from this squid for stable isotopes will indicate an unusual feeding history. Males were taken throughout the area sampled (Fig. 5) while females, as in the past, were taken almost exclusively near the subtropical front (Figs. 2, 6). Indeed, the station right on the front captured three times as many females (17) as any station during the 4-year study. Males were also captured in record numbers there (38). A subsequent station near the front (SST = 20.5° C) was making good catches until a large billfish appeared and the squid disappeared for the remainder of the night. The weak trend in size of males relative to latitude seen in some previous years was also apparent in 1999 (Fig. 7). These data were not very consistent due to the large disparity between stations in numbers captured. We now have sufficient data, however, to eliminate all stations with fewer than nine captured males. The combined data set shows a size trend with a low R^2 value (0.31) which is, nonetheless, significant (p=0.05) (Fig. 8). The southern captures tend to be slightly larger, perhaps reflecting the time taken for the southerly migration or simply the longer residence time in these waters that allowed growth.

Distribution of paralarvae

The distribution of red-squid paralarvae was consistent with that of previous years (Fig. 9). Paralarvae are generally well south of the subtropical front. Over the four years of sampling, captures of paralarvae at six stations exceeded mean values of one per 25m^2 of ocean surface. The surface temperature at five of these stations,which were broadly spread longitudinally (14°)

but restricted latitudinally (2.5°), was within a tenth of a degree of 22° C and, therefore, agrees with our previous work (Bower, 1995) that suggests a spawning preference between 21 and 24° C. The sixth station was off Nihoa at 24.1° C. Our assumption that large, mature females are present south of the front (where spawning presumably occurs and where males are common) but occupy deeper waters than to the north and therefore are not suseptible to our typical fishing methods, received limited support by the deep capture of a single female off Nihoa. (An alternative hypothesis that fails to account for the distribution of males is: Spawning occurs in deep water beneath the subtropical front and drift there carries the very slowly developing (due to cold temperatures) embryos south to the broad nursery grounds.)

Feeding

The initial processing of *O. bartramii* stomachs from 1999 has been completed. This includes weighing stomach contents and sorting out otolith and squid beaks, hooks, and gladii. Because of the apparent abundance of *O. bartramii* near the subtropical front, we suspect that this is a good feeding grounds. However, when we examine the body-length normalized stomach-content weights normalized against body weight and compared this with distance from the front, clear trends are not apparent (Fig. 10). When we attempt to remove the bias of aberrant feeding around the ships lights by removing undigested material from the data, the values for some stations are reduced but the overall trend doesn't change much (Fig. 11). If we look at the number of fish eaten (as estimated from otoliths and eye lenses), the number seems to increase with increasing latitude within an individual year but this has not yet been tested statistically (Fig. 12).

Identification of the stomach contents progresses more slowly. We continue to build our reference collections of otoliths and beaks, which are now quite extensive. Analysis of the otoliths found in squid stomachs reveals 20 identifiable families of mesopelagic fishes. Roughly 10% of the otoliths found are either unknown or unidentifiable. Fish of the family Myctophidae make up 35% of the total otoliths found. For the 1999 cruise, the otoliths found in the closely related squid *Sthenoteuthis oualaniensis*, which is common around the main Hawaiian Islands but has decreasing abundance north of the Islands, were also analyzed (Fig. 13). This squid seems to feed even more heavily on Myctophidae than does *O. bartramii*. Within the Myctophidae, however, *O. bartramii* and *S. oualaniensis* may feed on different species (Fig. 14). The distribution of *S. oualaniensis* overlaps that of *O. bartramii* during the winter, and it is a potential prey as well as competitor of *O. bartramii*. *S. oualaniensis* is the basis of a small commercial fishery around the Hawaiian Islands. We study *S. oualaniensis* in order to understand the prey/predator/competitor interactions with *O. bartramii* in the area of spatial overlap.

Data on the squid in the diet of *O. bartramii* based primarily on beaks and beak fragments found in stomachs is also progressing. At present 14 families and 20 genera of epipelagic and mesopelagic cephalopods have been identified in stomach contents. Of the beaks examined (cruises 1997 and 1999), 12% are presently unidentifiable, 8% have probable identifications and 80% have been identified to genus at least. The dominant prey in stomachs of both *O. bartramii* and *S. oualaniensis* is *Onychoteuthis* spp. Three species of *Onychoteuthis* occur in these waters but species identification of beaks to the species level in this genus has not yet been accomplished.

While the otolith and beak analyses are yielding reproducible data on what types of prey the squids are feeding on, the data are only in the initial phases of analysis. The analysis will be done by Matt Parry as a major component of his dissertation.

Stable isotopes

We have begun to examine the stable isotopes of *O. bartramii* which will provide a measure of the trophic position of this squid that is independent from that determined by stomach content analyses. In addition stable isotopes encased in secreted structures have the potential of revealing the past history of feeding. Our first set of isotopic data indicate that separation of major trophic levels in the open ocean using delta ^{13}C and ^{15}N isotopes is possible (Fig. 15). Results from analyses on meso-zooplankton indicate that there may be features associated with the subtropical front that may affect the isotopic signal in the food web (Fig. 16). These data also show that, in the squids *O. bartramii* and *S. oualaniensis*, these isotopes vary as a function of mantle length but more so for nitrogen than carbon especially in *O. bartramii* (Figs. 15, 16). For both squids delta ^{13}C is low in squid paralarvae, high in juveniles, then low in adults (Fig. 17). Separation of age from sex in the trends is still incomplete. Delta ^{15}N appears to increase linearly with size in *S. oualaniensis* (Fig. 18). In *O. bartramii* delta ^{15}N seems to increase up to a certain size, then remain constant or even decreases slightly at large-sized females; however, statistical analyses are needed. While these data are very encouraging, proper interpretation will require more samples that examine a variety of tissues, ages, sex, and geographical regions.

Reproduction

The relatively large number of mature females taken in 1999 (25) has given us a clearer picture of the relationship between the oviduct with its cargo of eggs, and the size of the nidamental glands, which secrete the bulk of material that forms the egg mass during spawning. Grouped together all females captured during this program can be fitted to a power curve with an R^2 of 0.85 (Fig. 19). This is a far better fit than that between the nidamental glands and the body weight or between the nidamental glands and the gonad weight. This highly significant relationship ($p < 0.001$) indicates that the size of the nidamental glands covaries with the fullness of the oviducts, as one would suspect since both are involved in spawning. As a result, the nidamental glands may contain histological clues to whether or not multiple spawning occurs. We have wanted to examine multiple spawning for some time but lacked resources to do so. We will now make this a priority.

Coordination with related programs

We continue maintain close working relationships with NMFS, especially with Mike Seki.

Request for Supplemental Funding

Our project was funded for two years, although the project technically extends for five years. We plan to continue studying the red squid for the full five years, that is through 2001. We have an agreement with Hokkaido University, Japan that makes their ship HOKUSEI MARU available for this period. The 1999 cruise was funded by PFRP through supplemental funding of somewhat lower magnitude than requested here for the year 2000 cruise. We have also requested funding for the year 2000 cruise through the International Programs of NSF. We are not confident, however, that this will be approved as our qualification for funding under their guidelines is questionable. If NSF funding is approved, we will reduce our request from PFRP accordingly. Here we request funds to pay for meals aboard ship (while personnel on ships in the U.S. academic fleet are not charged for meals, they are on Japanese ships) and to pay for additional stable-isotope analyses.

Last year PFRP provided funds to examine the use of stable isotopes in evaluating the trophic ecology of the red squid. The initial results of this aspect of our study have been presented above. We are pleased with the data thus far and optimistic about the potential of this technique. The stable isotope data are being processed and analyzed by M. Parry as the second part of his dissertation.

The first runs of analyses for stable isotopes consisted of 110 samples, including standards and blanks. Many more samples are needed examine the effects of different tissues, sizes, sexes, localities, years, as well as the possibility of retrospective analyses . In addition, the data from the red squid must be placed in context with other major species in the ecosystem, especially predators, prey, and competitors. With a minimum of about six samples/tissue/animal needed for statistical comparisons, the number of samples and costs mount quickly. Processing samples takes considerable time and is an even more important limiting factor. As a result, we have carefully planned the minumum number of samples needed and have arrived at a figure of 950 . Presently we have funds for about 200 additional samples. We therefore request funds for 750 samples in order to complete the project. There should be no problem in obtaining the necessary squid, squid prey, and some competitor samples (e.g. *S. oualaniensis*) during the year 2000 cruise. Samples from predators and large competitors can be obtained from NMFS (Seki, personal comm.). Therefore, we anticipate that the 2000 cruise will be the final cruise for collecting data on stable isotopes. We also will have sufficient stomach content data after 2000 to terminate collecting on that portion of the project as well.

BUDGET: YEAR 4: 1999-2000

Food costs at sea ($20/day/person) for 10 people.*	$3,000
Miscellaneous supplies for year 2000 cruise (U.H van rental for loading and unloading ship, fishing line, preservatives, rope, etc.)	$ 500
Stable isotope analyses (750 samples at $10/sample)	$7,500
Overhead at 25%	$2,750
Total	$13,750

If NSF funds are approved, the total will reduce to $9,375.

*We normally take 10 people on our cruises to help with jigging for red squid with fishing poles (most female red squid are caught by this method), and with other aspects of the research.

General Project Synopsis

This project examines the general ecology of the red squid *Ommastrephes bartramii* in the central North Pacific near the Hawaiian Archipelago. Our interest in the red squid is based on (1) its considerable potential for a Hawaii-based commercial fishery and (2) its importance in the ecology of the broadbill swordfish, which is a major commercial fishery in Hawaii. This project involves strong cooperative aspects with the National Marine Fisheries Service (NMFS) in Honolulu and Hokkaido University in Japan.

We must have effective sampling methods to study this squid. For catching adult squid, we have experimented with large trawls, pole and line jigging, automatic jigging machines, and squid drift-lines. None of the methods have been fully satisfactory in catching large numbers of squid. We are uncertain whether the problem lies in the methods themselves or in the distribution of the squid. At present, most of our sampling is done aboard the FTS HOKUSEI MARU from Hokkaido University, although we have obtained samples from commercial fishermen and NMFS. For sampling paralarvae, we use standard plankton nets aboard the HOKUSEI MARU.

At present we are trying to obtain data on latitudinal and other environmental trends in feeding, reproductive condition, and abundance. Our data, although limited, suggest that adult squid are most abundant in the vicinity of the subtropical front. Females, unlike males, are difficult to capture south of the front. We suspect this is due to a nighttime habitat that is deeper in the south than near the front and this makes females less vulnerable to our sampling gear.

Paralarvae are caught predominantly south of the front, with a maximum in abundance near SST of 22°C, but their distribution is patchy and widely scattered there. Apparently the spawning grounds of this squid constitute a broad area south of the subtropical front. In the Hawaiian region, the red squid feed predominately on midwater fishes and other squids. There is some indication that supplemental feeding occurs during the daytime in deep water and that food is more abundant near the subtropical front. Our present evidence suggests that the red squid is a multiple spawner and that spawning is fueled by feeding on the spawning grounds.

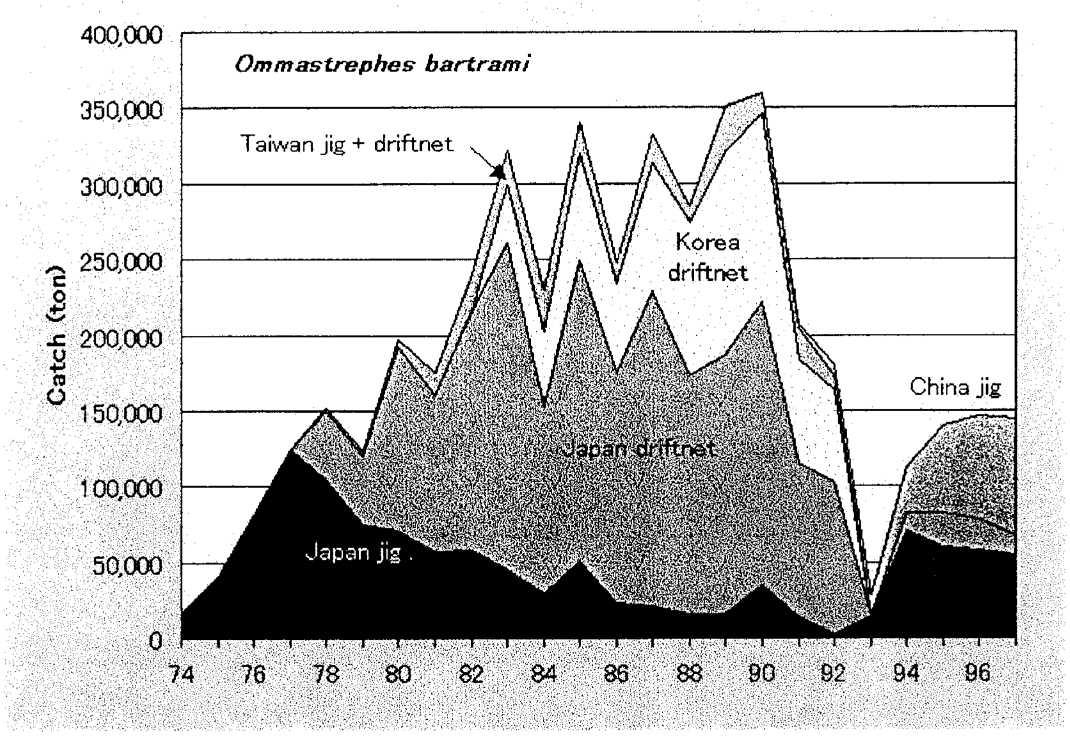


Fig. A. North Pacific commercial catch data for the red squid, *Ommastrephes bartramii*, from 1974-1997. From: http://www.enyo.affrc.go.jp/sqd-sec/sqdstat.html

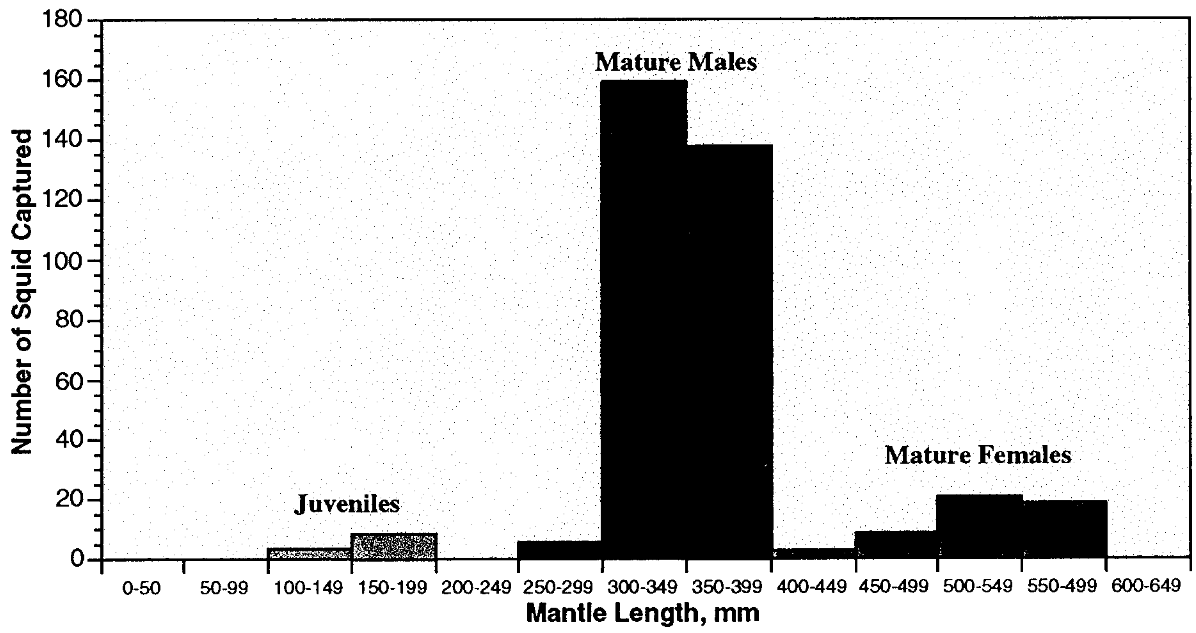


Fig. B. Catch data for the red squid, *Ommastrephes bartramii*, during this PFRP study from 1996-1999.

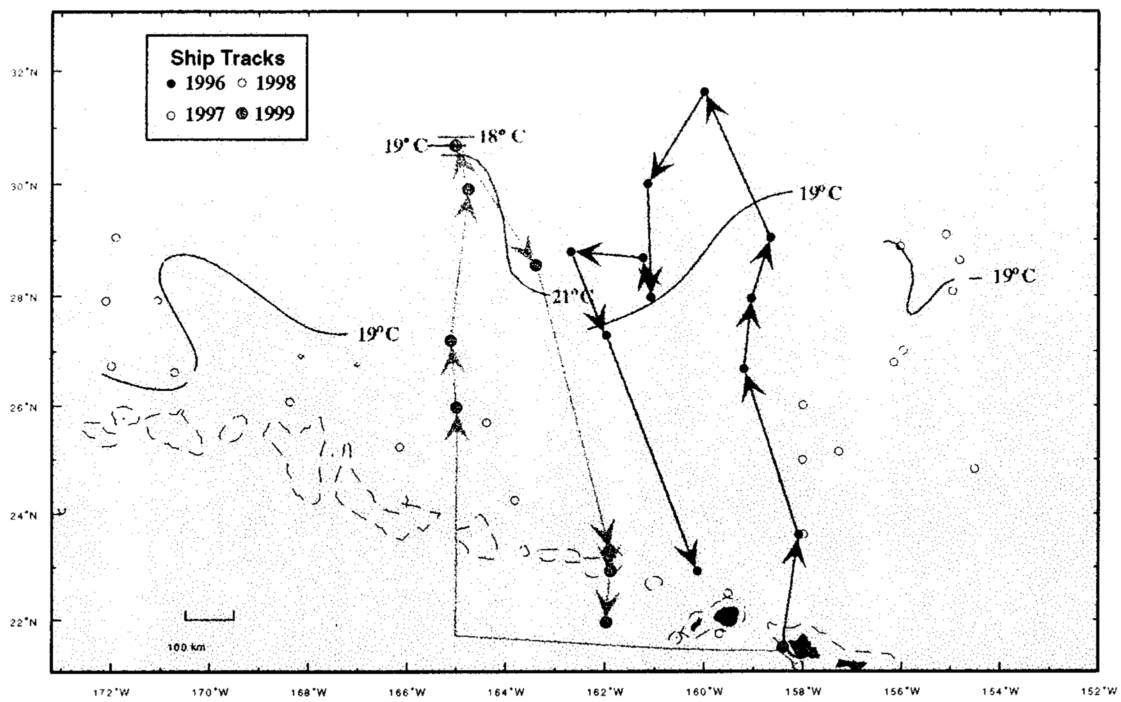


Fig. 1. Geographical plot of the cruise tracks of the FTS HOKUSEI MARU for cruises of February 1996, 1997, 1998 and 1999. Positions of major oceanographic stations are indicated by filled circles. The 19° C surface isotherm approximates the position of the subtropical front.

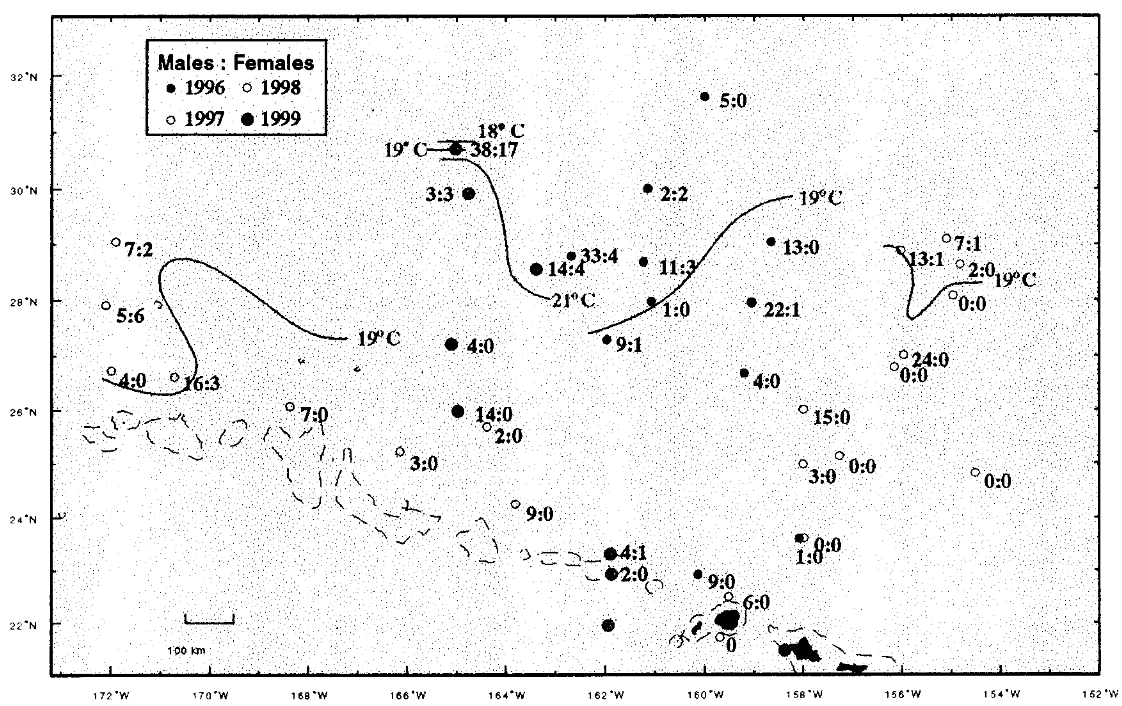


Fig. 2. Geographical plot showing the catch of male, compared to the catch of female *O. bartramii* at each station for February 1996, 1997, 1998 and 1999.

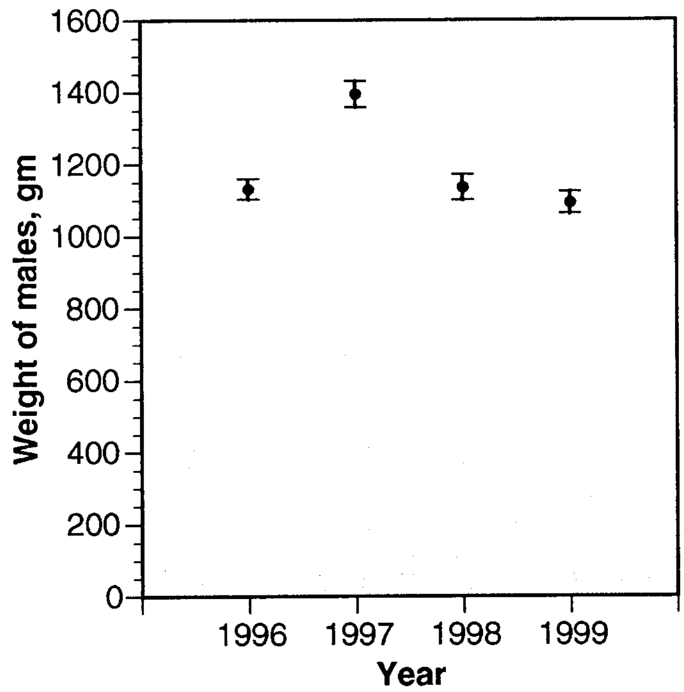


Fig. 3. Mean weights and confidence limits for male *O. bartramii* for February 1996, 1997, 1998 and 1999.

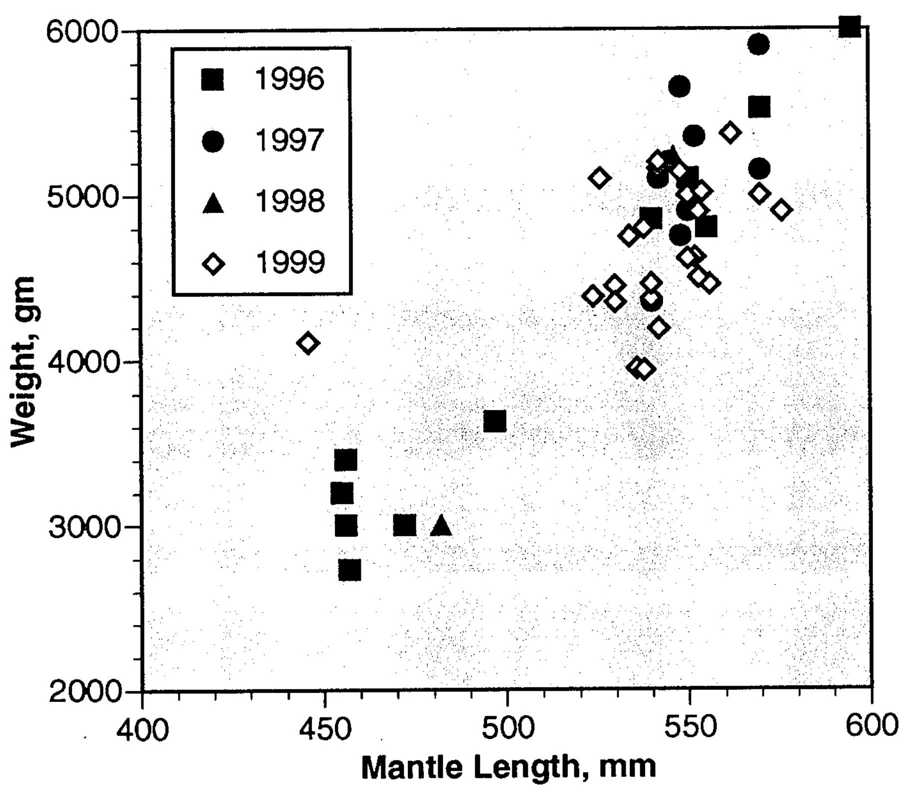


Fig. 4. Length-weight plot of female *O. bartramii* comparing size for February 1996, 1997, 1998 and 1999.

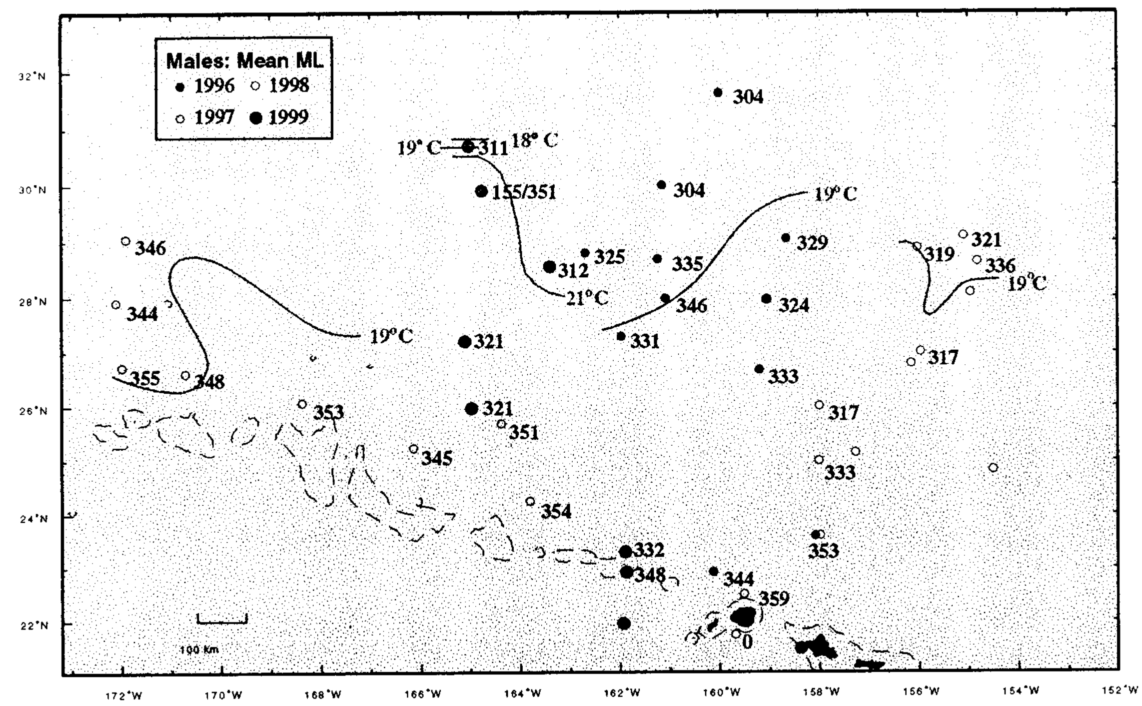


Fig. 5. Geographical plot showing the mean mantle length per station for male *O. bartramii* in February 1996, 1997, 1998 and 1999.

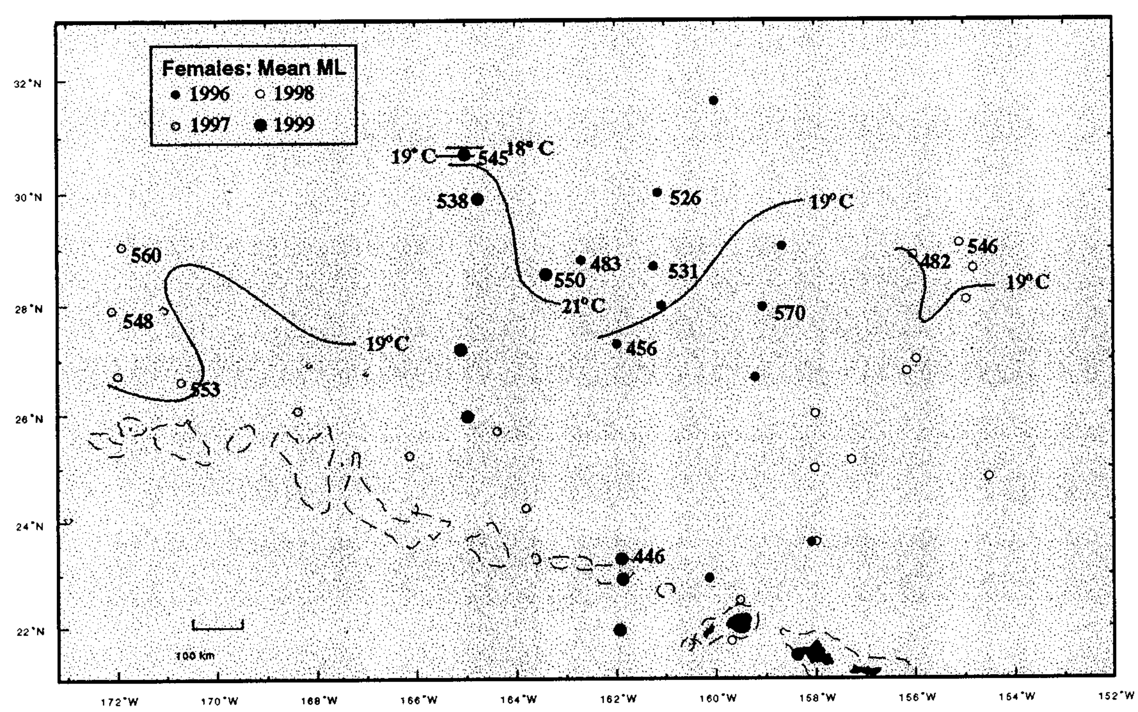


Fig. 6. Geographical plot showing the mean mantle length per station for female *O. bartramii* in February 1996, 1997, 1998 and 1999.

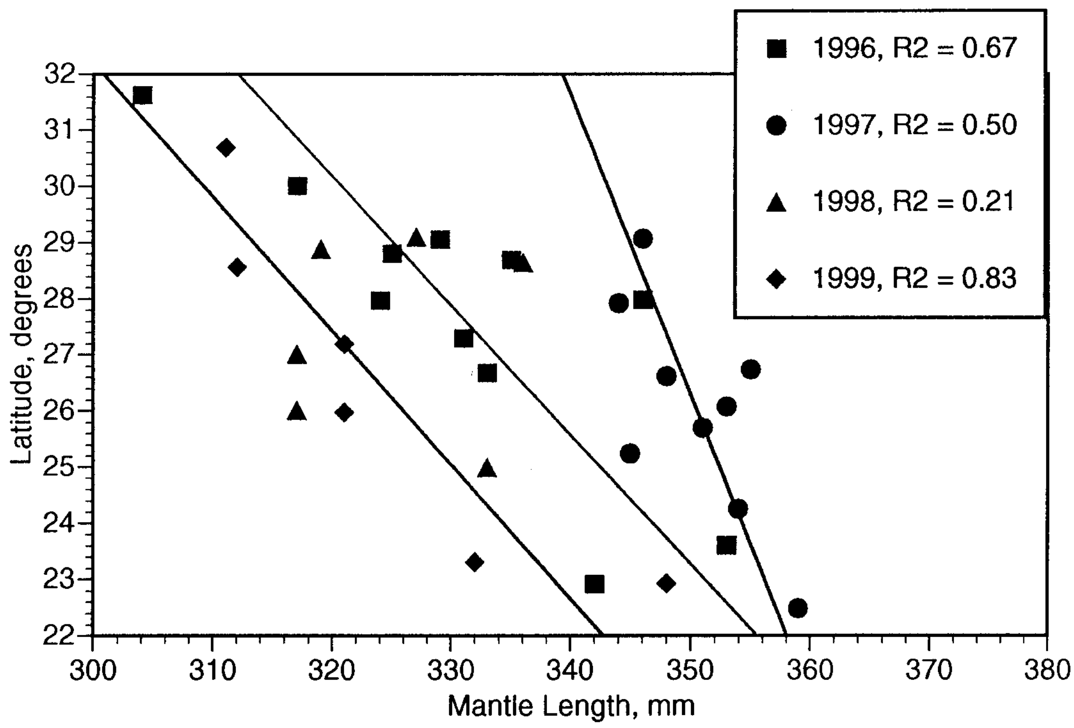


Fig. 7. Graph showing the change in mantle length with latitude for *O. bartramii* males for years 1996, 1997, 1998 and 1999 based on stations catching one or more males.

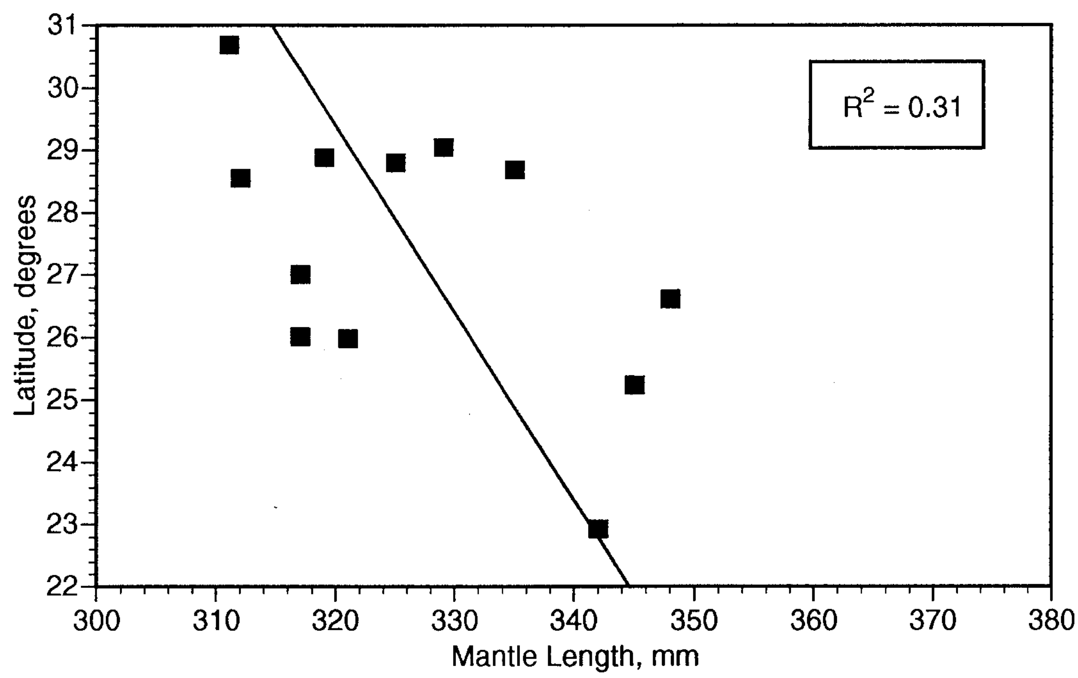


Fig. 8. Graph showing the change in mantle length with latitude for *O. bartramii* males for years 1996-1999 based on stations catching 9 or more males.

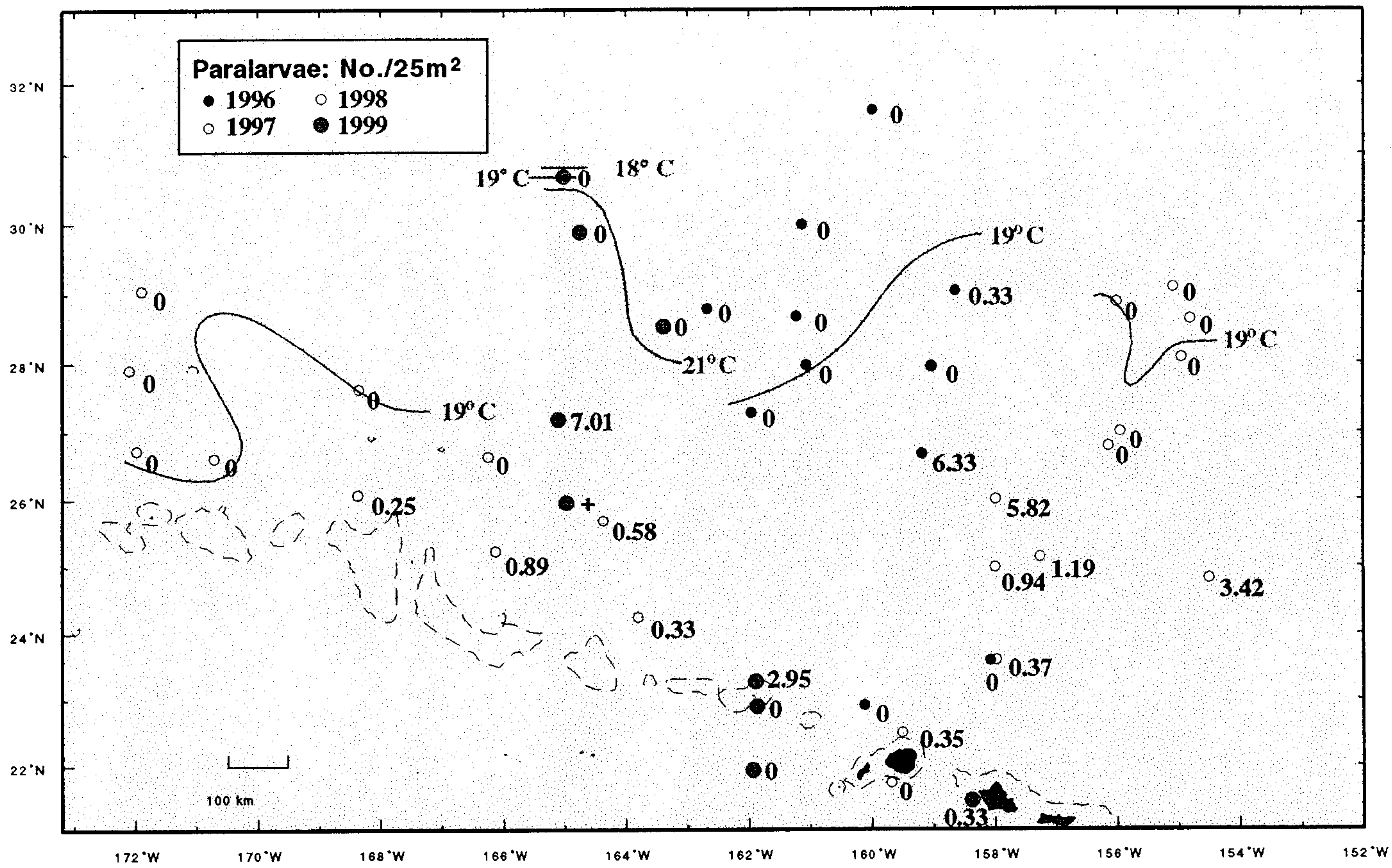


Fig. 9. Geographical plot showing catch rates of *O. bartramii* paralarvae per 25 m^2 of the ocean surface in February 1996, 1997, 1998 and 1999. The approximate area sampled per tow is 25 m^2. + - Paralarvae present in surface tows but oblique tows not taken due to poor weather conditions.

Fig 10. Plot of median weight *O. bartramii* stomach contents per station of normalized to ML [(content wt. in g * 100)/ML] vs distance from the 19°C SST isotherm. All stomachs included, negative distance values represent southern direction from the isotherm.

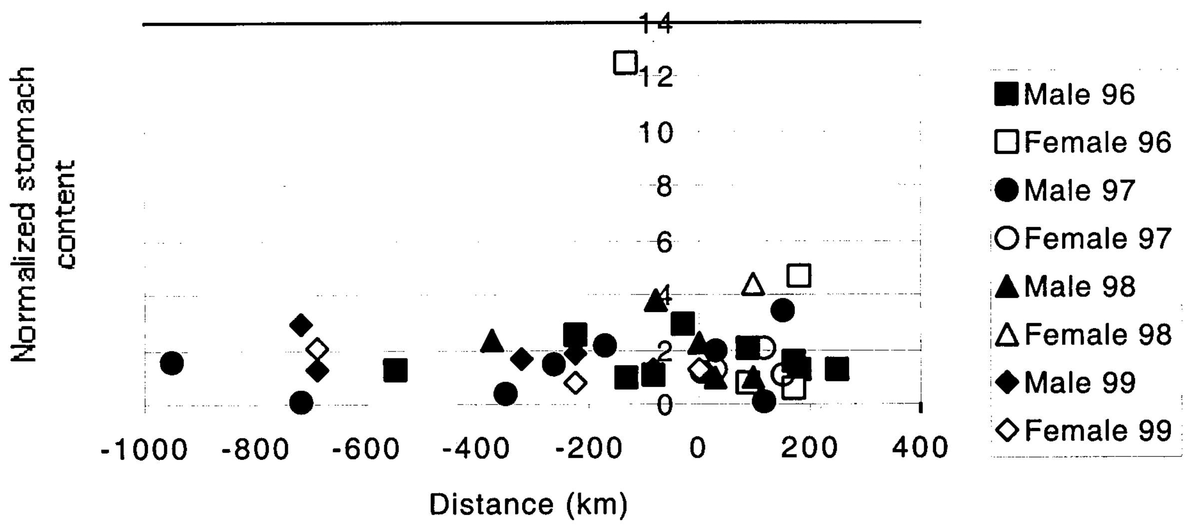


Fig 11. Plot of median weight *O. bartrami i* stomach contents per station of normalized to ML [(content wt. in g * 100)/ML] vs distance from the 19°C SST isotherm. Undigested material removed negative distance values represent southern direction from the isotherm.

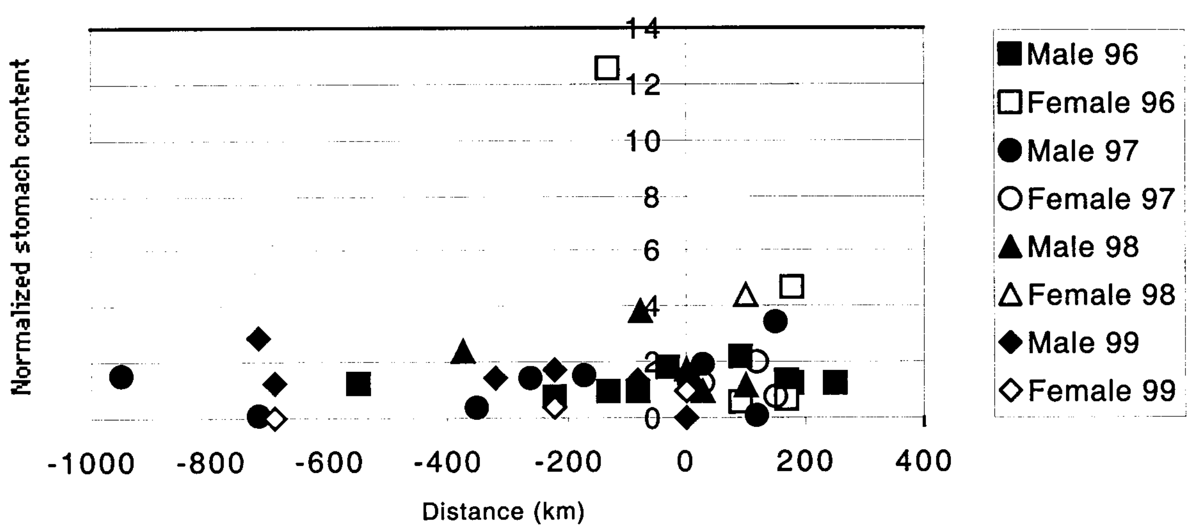


Fig. 12. Plot of mean number of fish eaten per station vs distance from 19° C SST isotherm for *O. bartramii*. Number of fish estimated from # of otoliths (sagitta) or eye lenses.

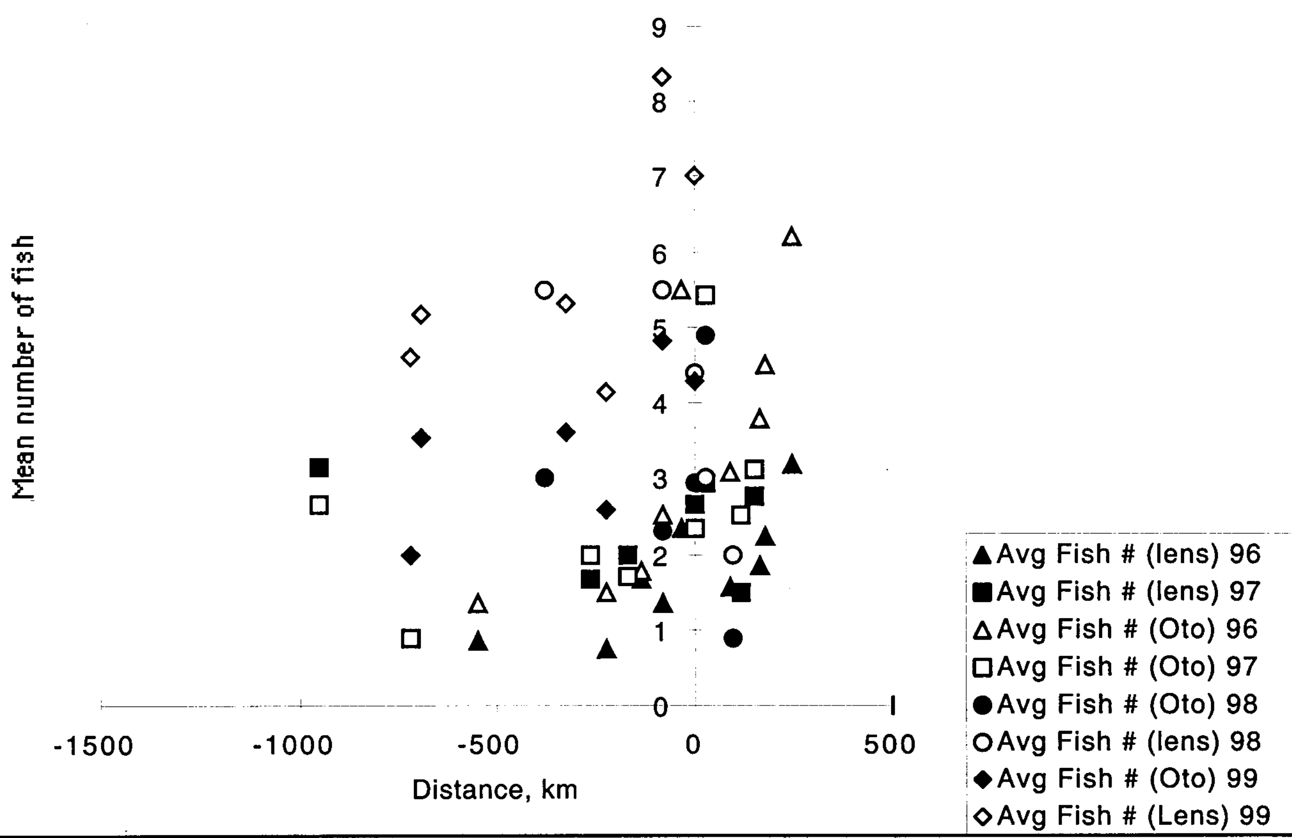


Fig. 13. Relative occurrence of otoliths of five major fish families from stomachs of *O. bartramii* and *S. oualaniensis*.

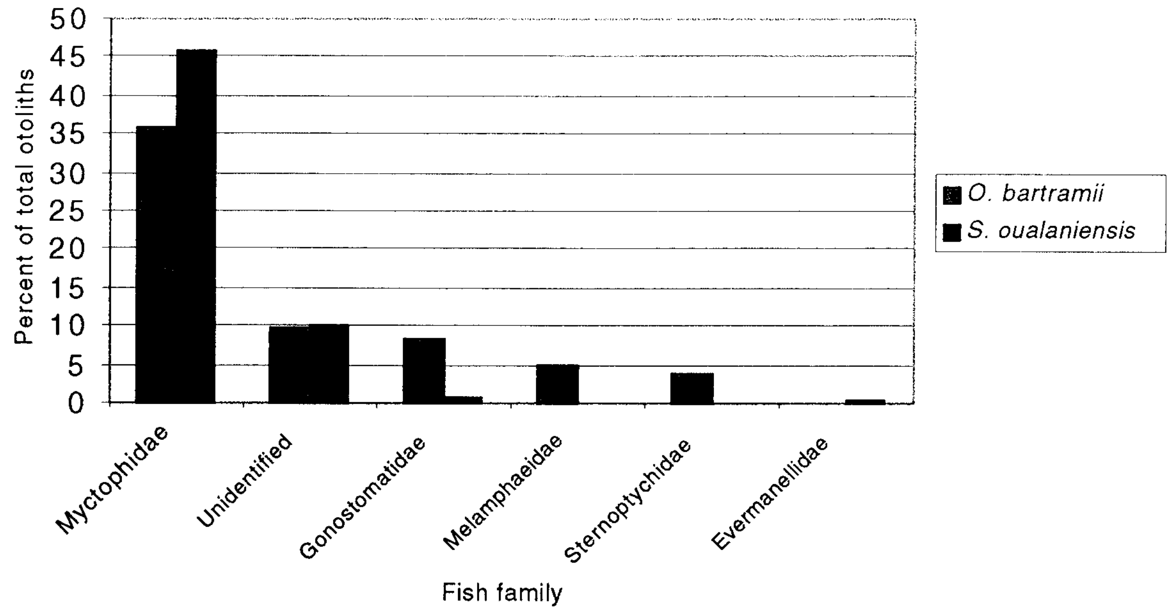
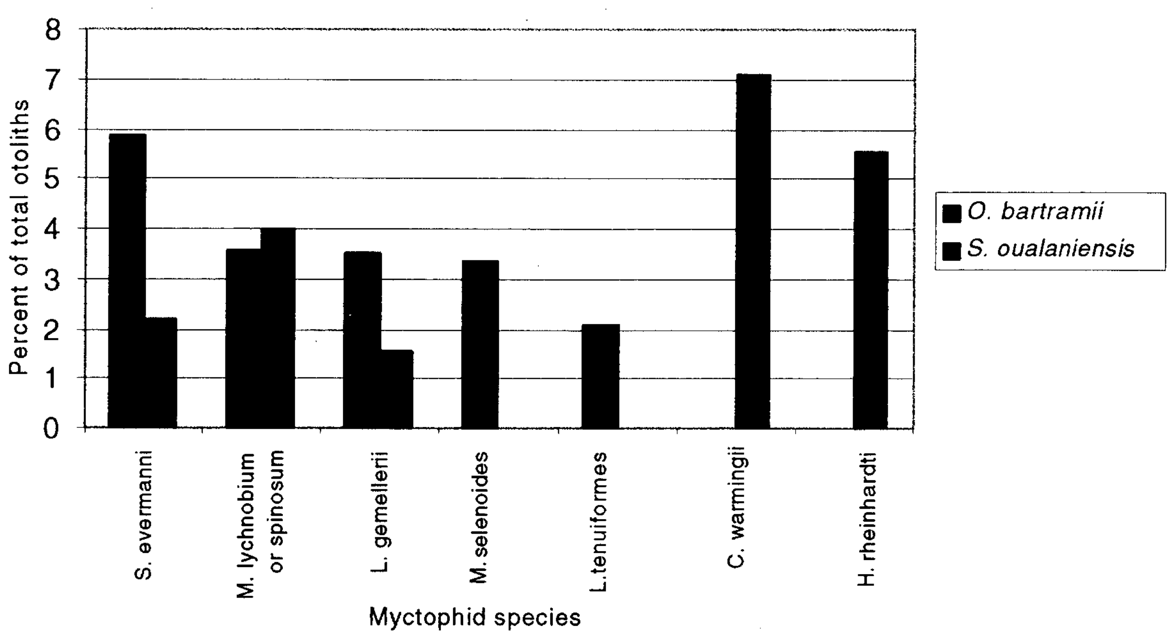


Fig. 14. Relative occurrence of otoliths of five major myctophid species from stomachs of *O. bartramii* and *S. oualaniensis*.



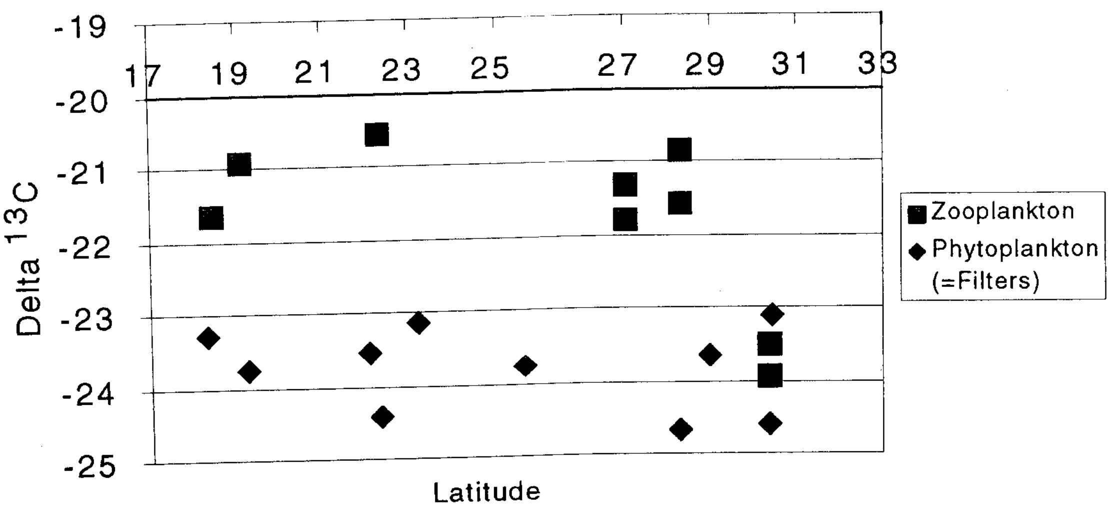


Fig. 15. Plot of delta ^{13}C vs latitude for zooplankton and filter samples

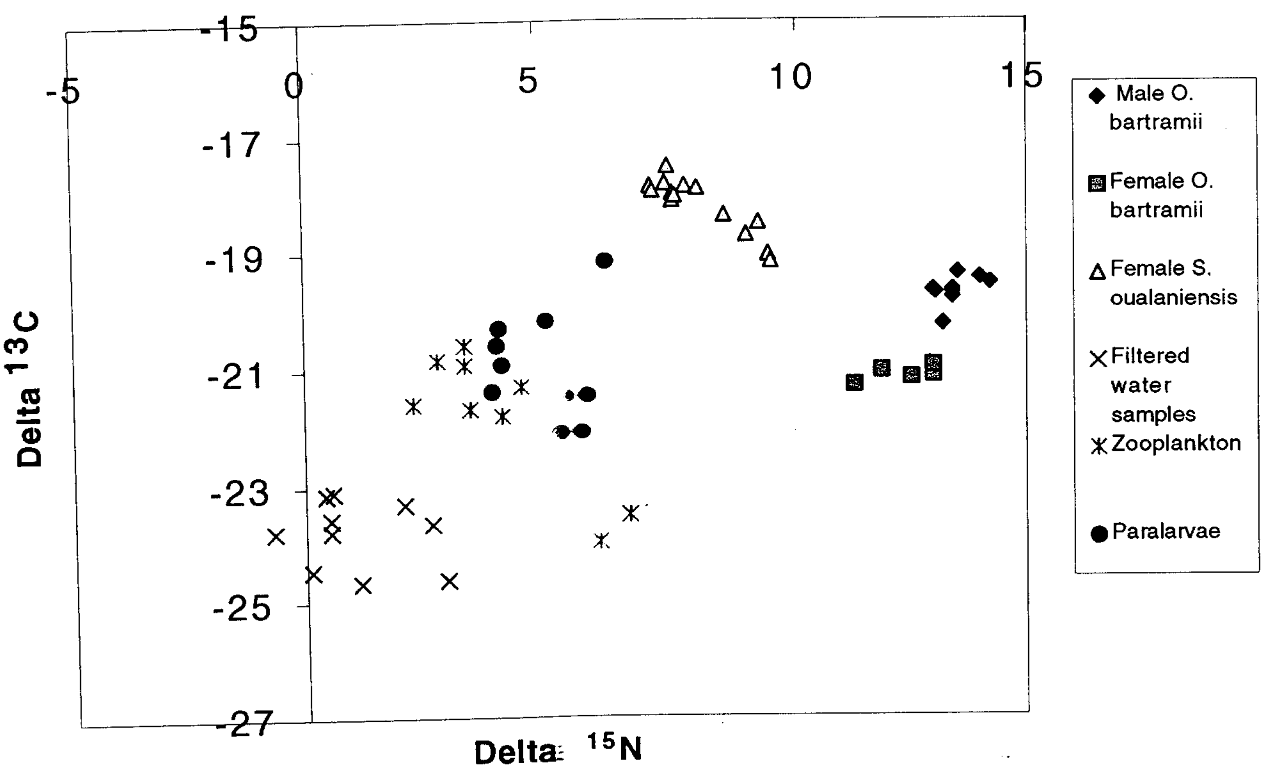


Fig. 16. Plot of delta 13C vs delta 15C for squid, zooplankton, and phytoplankton (=filter) samples.

Fig. 17. Plot of delta ^{13}C vs mantle length for mantle muscle of two squids

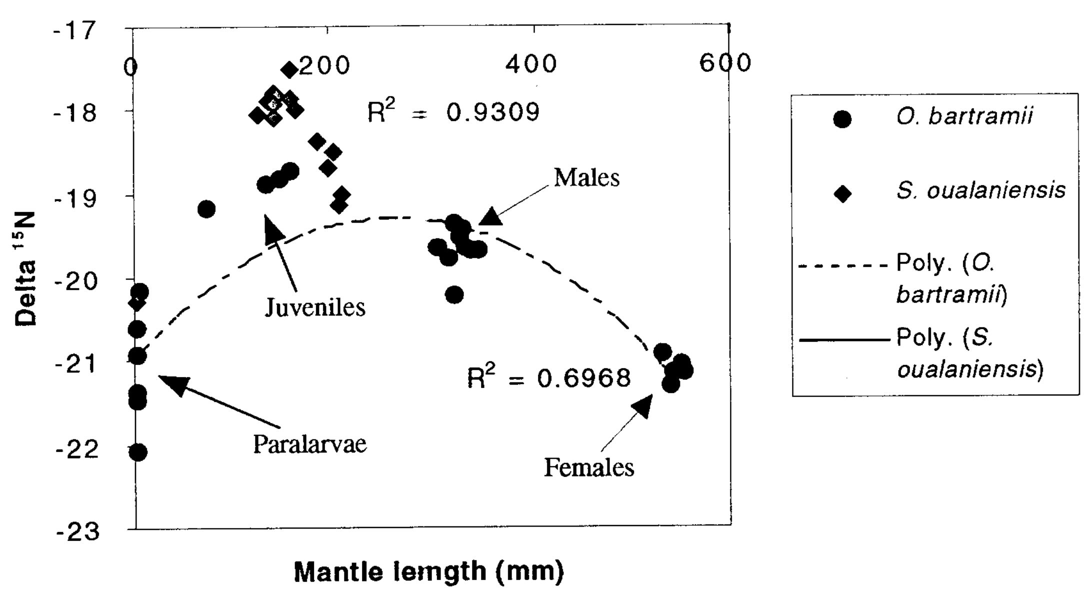
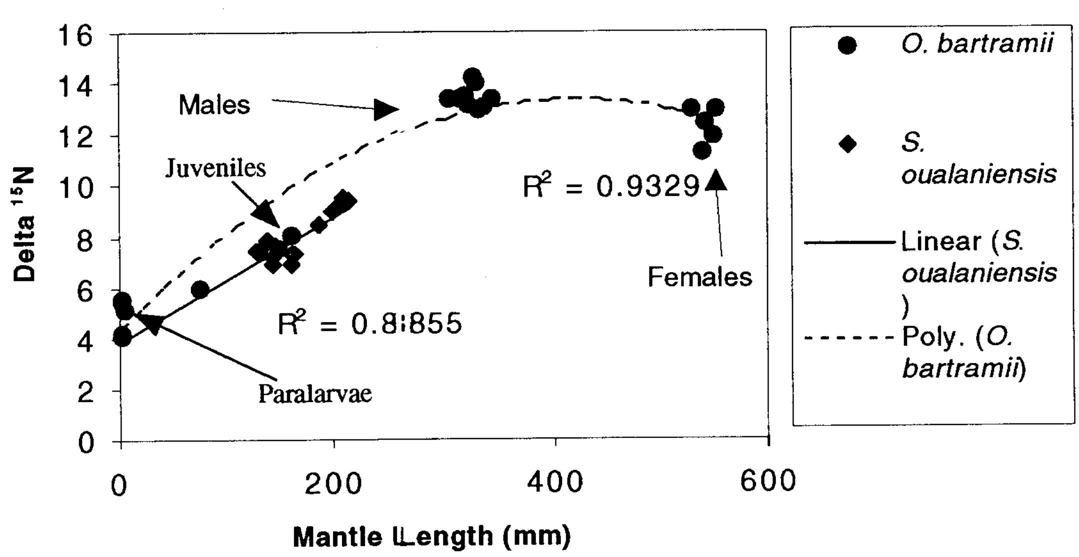


Fig. 18. Plot of delta ^{15}N vs. mantle length for mantle muscle of two squid species



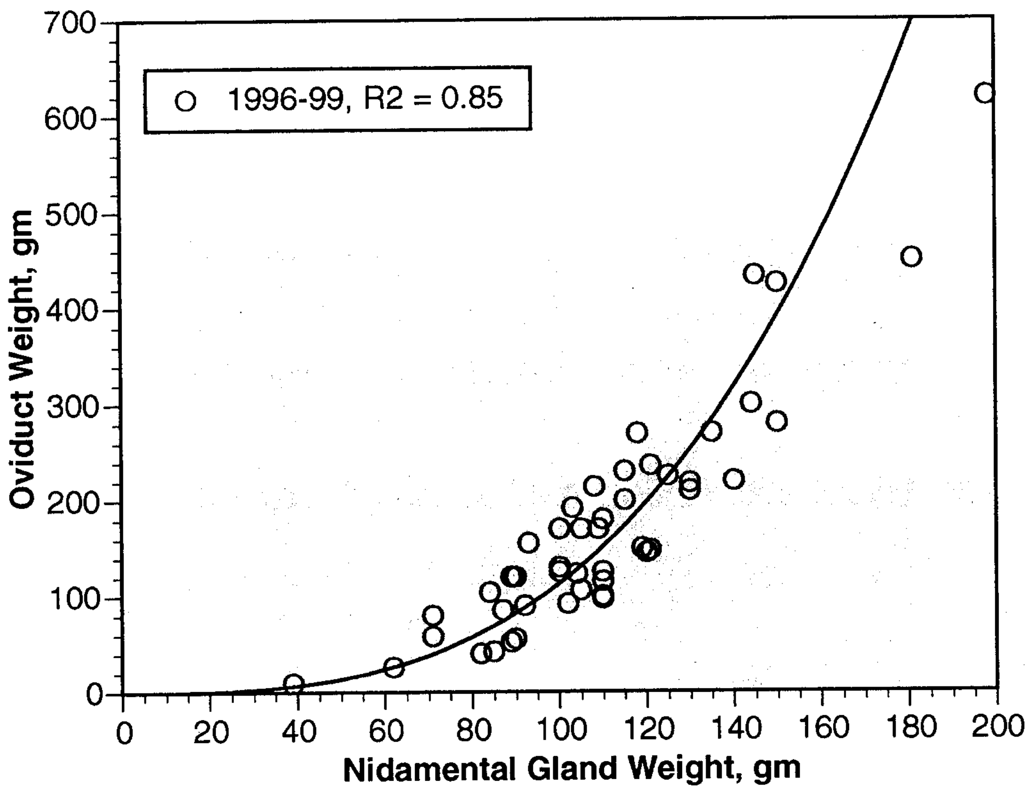


Fig. 19. Relationship between oviduct weight and nidamental gland weight for female *O. bartramii* captured from 1996 through 1999.