Conclusions

After this experience it can be said that Ketamin was a safe anaestetic in this walrus and that the fibreoptic gastroscope is a very helpful instrument for the investigation of the upper digestive tract.

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INVESTIGATIONS ON THE VIABILITY OF LARVAL HELMINTHS AFTER FREEZING *

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Summary

Viability studies carried out on larval helminths, particularly those of the phylum Nematoda, under freezing conditions, have yielded a variety of results. PARFITT (1971) and CAMPBELL, et al. (1973) using liquid nitrogen (-196°C) and quick freezinn methods found a high infectivity in sheep nematodes after prolonged freezing times (up to 44 weeks). TURNER (1953) ran year-round field trials on *Nematodirus spathiger* larvae and found a 37% survival rate after 10 months at 28°-30°F.

This study was conducted on four species of fish *(Allosmerus elongatus, Thaleichthys pacificus, Clupea harengus, Trachurus symmetricus)* and one species of squid *(Loligo opalescens)* taken from the frozen food locker at the NUC bioscience facility, San Diego, California. All five species are currently being utilized as food for marine mammals at the facility. Larval helminths were recovered and observed for viability from a sample of each of these species to elucidate the possibility of research animals being infected in captivity under present feeding procedures.

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Materials and Methods

Frozen samples were taken from the NUC freezer and transported in insulated containers to a laboratory freezer. Samples of five each were thawed at ambient temperature (22°C), measured, sexed (fish only) and Baermanized in acidulated pepsin solution at 37°C (CABLE, 1958). The Baerman fluid and digested residue (after 24 hours) were examined for larvae and any associated activity. The artificial digestive fluid was checked for results by exposing freshly killed fish at 37°C. Activity rate of recovered helminths in this control sample was nearly 100%. Representative helminth samples were fixed in AFA (10 parts formalin, 50 parts 95% ETOH, 2 parts glacial acetic acid and 40 parts distilled water) and mounted for future identification. Nematodes were cleared in glycerin jelly. All other helminths were stained in Semichron's carmine cleared in xylene and mounted in Piccolyte.

Results

Squid *(Loligo opalescens)*

State Fish Company recorded no lot numbers on squid so total duration of freezing time is not available. It is estimated at 180-240 days. Sample was frozen at -40°C for 24 hours, stored at -10°C, transported at 0°C. Storage at the NUC facility is reported as 0 to +3°C and laboratory storage was at -4°C. All Baerman fluid for squid samples were negative.
Remarks: The fact that the squid samples were negative does not indicate that no larvae were present. It indicates that no viable, living larvae were found in the Baermanized sample. Unencysted, dead larvae are often digested in tissue and do not appear in the sediment.

Herring *(Clupea harengus)*, Table 1.

Herring sample (lot 7884) was frozen 1/2/73 by State Fish and examined 8/11/73. Only nematodes were recovered.

TABLE 1.

No.	Sex	Length	Time Frozen	Parasites Recovered	Total Parasites Recovered	Active Parasites
H-1		27.6 cm	221 days	*Anisakis* sp.	7	2
				Contracaecum sp.	5	0
H-2		26 cm	221 days	*Anisakis* sp.	4	0
				Contracaecum sp.	5	0
H-3		23.4 cm	221 days	*Anisakis* sp.	3	0
				Terranova sp.	1	0
H-4		26.8 cm	221 days	*Anisakis* sp.	3	1
				Contracaecum sp.	2	0
H-5		22.7 cm	221 days	*Contracaecum* sp.	2	0

Remarks: Of the 32 parasites recovered only 3 (9.6%) *Anisakis* sp. were found demonstrating any activity. This activity consisted of slight tail movements for a short duration (1-5 min). Infectivity of these larvae is extremely doubtful.

Jack Mackerel *(Trachurus symmetricus)*, Table 2.

The Mackerel sample (lot 8662) was frozen 6/11/73 and examined 9/14/73.

TABLE 2.

No.	Sex	Length	Time Frozen	Parasites Recovered	Total Parasites Recovered	Active Parasites
M-1		33.3 cm	95 days	Nematoda		
				Anisakis sp.	3	0
				Cestoda		
				Trypanorhyncha	50+	0
M-2		19.2 cm	95 days	Nematoda		
				Anisakis sp.	2	0
				Cestoda		
				Trypanorhyncha	1	0
M-3		31.6 cm	95 days	—	—	—
M-4		20.4 cm	95 days	—	—	—
M-5		32.0 cm	95 days	—	—	—

Remarks: No activity was observed in any of the larval helminths recovered from Mackerel. The class Trypanorhyncha are cestodes found as adults in elasmobranch fishes. The trematode recovered in M-2 was partially digested and unidentifiable.

Silver Smelt *(Thaleichthys pacificus)*
The Silver Smelt sample (lot 8761) was frozen 7/2/73 and examined 9/22/73 (81 days). Of the five entire fish digested (all male, average length 19.6 cm), two *Contracaecum* sp. ware recovered from one specimen (S-1). No activity was observed.

White Bait Smelt *(Allosmerus elongatus)*
The White Bait sample (lot 8522) was frozen 3/15/73 and examined 10/12/73 (190 days). This sample consisted of 3 females and 2 males averaging 74.1 cm. Two specimens (WBS-2, WBS-5) contained encysted larval acanthocephalans of the genus *Corynosoma* in the mesenteries. Although this genus is a common parasite in marine mammals (primarily pinnipeds), no movement was observed in the recovered parasites indicating a lack of infectivity.

Discussion

The results of this study seem to preclude the possibility of infectivity in NUC research animals under ourrent feeding practices. There are many variables to be considered in larval survival under freezing conditions (a. type of freezer employed, b. the medium surrounding the sample, c. the size of sample, d. the rate of cooling, e. the age of the larvae, f. individual differences in larvae, g. host and tissue occupied).

Of these factors the size of the sample, temperture and time exposed appear to be the most important. In a study by GUSTAFSON (1953), he concludes that 100 lb blocks exposed to -17°C to -30°C for 24 hours is adequate to kill encysted heterocheilids (Anisakis group).

Under the present handling, i.e. State Fish Co. freezing at -40°C for 24 hours, storage at -10°C, transported at 0°C and stored at NUC at 0 to +3°C, it appears any viable larvae surviving the freezing condition would succomb to internal mechanical damage by ice crystal formation. Previous work by PARFITT (loc. cit.) and CAMPBELL (loc. cit.) demonstrate that flash freezing and storage at very low temperatures results in a high yield of viable larvae after thawing. Handling at varied higher temperatures, although it may be detrimental for food storage appears to be the most successful method of killing larval parasites.

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