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WORKING WITH LIVING KRILL – THE PEOPLE AND THE PLACES

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The fascination of Antarctic scientists with Antarctic krill and their capabilities has a long and varied history, and prompted many scientists to maintain and manipulate krill under laboratory conditions. Starting in the Discovery era with Mackintosh at the King Edward Point labs on South Georgia, 1930, scientists have collected krill from sailing vessels, small boats, inflatable zodiacs and large ice-breaking vessels. Krill have been maintained in small and large jars, deep rectangular tanks, large round tanks and in flow-through and recycling systems. They have been maintained both on board research vessels and in laboratories, in flowing seawater systems at ambient conditions and in temperature-controlled environmental rooms. A few researchers have transported living krill back to their home laboratories, for example tropical laboratories in Japan (Murano) and Australia (Ikeda), temperate laboratories (Nicol) in Australia, a northern European laboratory in Germany (Marschall) and a sunny maritime laboratory in California (Ross and Quetin). The goals have been varied: short-term experiments to understand *in situ* physiological rates, long-term experiments to test the effects of manipulations or controlled changes in environmental conditions, and behavioral responses. We take you on a brief historical tour as we trace the lineage of modern day research on living Antarctic krill.

Keywords: Antarctic; Krill; History; Field studies; Laboratory studies; Technology; Holding; Transporting

INTRODUCTION

The success of modern day research on living Antarctic krill (*Euphausia superba* Dana) is a result of multiple investigators testing techniques of collection, maintenance and transport of live euphausiids over the past eight decades. Scientists have used live euphausiids to achieve several goals. One important goal is the measurement of physiological rates – metabolic rates (oxygen consumption and ammonia excretion), growth and molting, feeding, spawning and egg production. Short-term experiments on physiological functions have been used to measure rates that reflect *in situ* conditions. Long-term experiments have allowed scientists to manipulate environmental

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conditions to answer questions about the capacity of this species and the source of variation in *in situ* rates. Questions about their functional response of feeding under different temperatures and food concentrations, and whether krill feed selectively and thus their impact on phytoplankton communities can be answered with these longer-term efforts.

A secondary group of questions concerned variations in development and growth under different food and temperature conditions, and the possible age of Antarctic krill. These were better addressed with rearing experiments, a type of long-term experiment. Also, questions on sensory sensitivity, mating, swimming and schooling behavior have been addressed by simulating *in situ* conditions and observing the behavioral responses of krill.

In this article we trace a partial history of the people, places and techniques of conducting research with live Antarctic krill from the early Discovery era in the Antarctic to the most recent success at the Port of Nagoya Aquarium in Japan (Hirano *et al*. 2003, Hirano and Matsuda, 2003). The story includes maintenance of live euphausiids at sea on ships, in the laboratory in the Antarctic, or transported to home laboratories for either experiments or culture. We focus on those krill scientists that have used living krill for experiments on rearing, growth and molting, and spawning. Those investigators that have maintained live krill for use in experiments on metabolic rates have not been included, due primarily to the relatively short duration needed for such experiments on board ship. Such experiments are critical to the establishment of a complete understanding of krill physiology, and our focus on longer-term experiments is not a function of their value. A review of research on other live euphausiids is outside the scope of this article. However, we would like to point out that work on live *E. pacifica* began in the mid-1960s (Lasker, 1966), and one example of more recent work on *Meganyctiphanes norvegica* (Buchholz, this volume) is included in this volume.

Early History – Discovery Era of 1930–1931

The brief of the Discovery Committee was to investigate the feeding and breeding habits of the whale and the distribution pattern of krill and plankton in the Falkland Islands (Coleman-Cooke, 1963; Anon, 1985). However, two small projects on living krill were subsidized by the Discovery Investigations. Dr. Neil Mackintosh and four other scientists went to South Georgia in late 1924 (Fig. 1) to help build a small marine station in King Edward's Cove, Grytviken, South Georgia, and study the carcasses of whales at the nearby whaling station. The original laboratory was known as Discovery House, and was the shore base for the oceanographic work and the research at the whaling station (Fig. 2). See web site at www.sgisland.org for additional information about South Georgia, and Mackintosh (1929) for a description of the marine station.

Although the results were not published immediately, the first attempts to maintain live Antarctic krill both at Discovery House and on board the *Discovery II* were in the 1930–1931 field season of the Discovery era. In December 1930, Mackintosh (1967) collected krill when they swarmed close to the surface at the jetty to the West of King Edward Point. He maintained individuals in ~4-L jars and changed water daily for up to 7 weeks to observe cycles of molting and growth (Table I). He subsequently found that krill were healthier in permanent dark or semi-dark, and put the jars in closed felt-lined boxes outside in the shade to keep them cool. Fraser (1936) (Fig. 3),

FIGURE 1 Mackintosh at far left in a string sextet with other men of the Discovery Investigation, ~1925–1926 season. Photograph from the library of photographs at the National Institute of Oceanography, Great Britain, taken by Dr. H. Matthews, and seen in Coleman-Cooke (1963).

FIGURE 2 Discovery House to the left with *Discovery II* moored to the jetty at King Edward Point, South Georgia, ~1931–1932 season. Photograph from the library of photographs at the National Institute of Oceanography, Great Britain, and seen in Coleman-Cooke (1963).

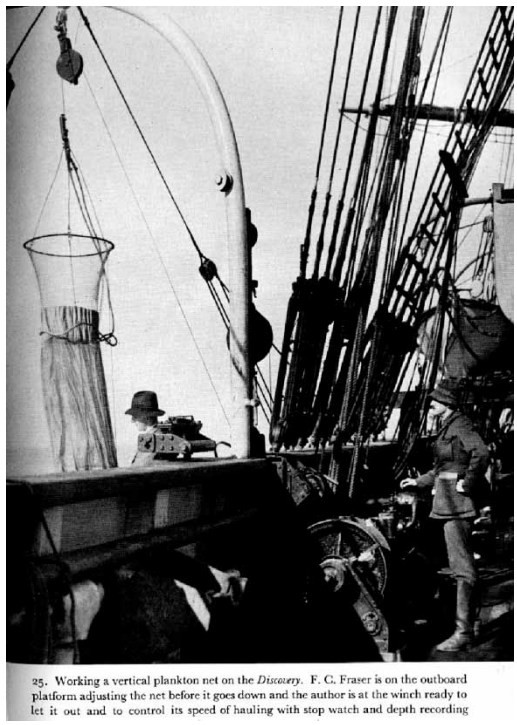

FIGURE 3 Fraser on the outboard platform of the *Discovery* adjusting the net before it descends (~1926). Photograph by Dr. S. Kemp, Plate 25 in Hardy (1967).

on board the *Discovery II* in December–February of that same season, briefly described his attempts to maintain gravid female krill and induce development of the released eggs (Table I). Although he used three different groups of gravid females, collected with nets from the ship, and controlled the temperature of some batches of eggs by surrounding the maintenance vessels with lumps of ice, development was never successful.

By 1931, finances were precarious (Coleman-Cooke, 1963), the Discovery Investigations closed, and Discovery House became a general store and carpentry shop for the British administration in South Georgia. Although the *Discovery II* sailed several more times to the Southern Ocean, with a final voyage in 1950–1951, no further reference was made to maintenance of live krill in the Discovery Reports. Thus, there was a 40-year hiatus before scientists once again maintained live Antarctic krill.

In 1968, the British Antarctic Survey (BAS) was asked to set up a scientific station at the former Discovery Investigation site, with terrestrial biology introduced in the 1969–1970 season and marine work in 1970–1971 (A. Clarke, personal communication). Everson and Clarke of BAS put a dry lab and a small aquarium back into Discovery House during the first summer. In the following year, better laboratories were built into the west end of the main accommodation building, and an aquarium built on the ground floor (A. Clarke, personal communication). In the summer of 1970–1971 Clarke (1976), encouraged by Everson to follow in Mackintosh's footsteps, also dip-netted Antarctic krill from swarms that appeared in King Edward Cove. He maintained individual subadult and adult krill (~25–40 mm in total length) in buckets of

TABLE 1 Maintenance conditions in Antarctic laboratories and on board ship for experiments with living Antarctic krill.

Investigator, Citation	*Volume Jar*	*Water change?*	*Light*	*Temperature Control?*	*Food*	*Max time*
Mackintosh (1967)	4-L per ind	Daily	Permanent or semi-dark better	Closed felt-lined box in shade	No supple	7 wk
Fraser (1936)	Aquaria on upper bridge	Unknown	Unknown	Varied, surround vessels with ice	No supple	8–9 d
Clarke (1976)	7-L per ind	Weekly	–	6″ flowing sw	No supple, green gut	>1 mo for 75%; ≤4 molts for 60%
Poleck and Denys (1982)	0.5–1.0 L per ind			0° and 5°C	Concentrated nat'l phytoplk	2 mo
McWhinnie and Denys (1978);	0.5–1.0 L per ind				Nat'l phytoplk	4 mo
McWhinnie et al. (1979); Denys et al. (1980)	600 to 1200 L (rectangular 0.6 and 1.2 m^3)	Flowing sea water, at 150 to 300 L h^{-1}		Flowing seawater, ambient	Ambient plus supple (cultures – sum '78) or lobster meat (win '78)	>12 mo
Buchholz (1985)	1.1-L Perspex	Flow-through aquarium	16L8D	3 ± 1°C	In situ from flow through	3 mo
Buchholz (1985)	1-L	Exchange sw via outlets	16L8D		Fed phytoplankton	
Ross and Quetin (1991);	– 2-L per indiv for IGR	None during expt	Dark	Flowing sea water table	No supple	4 d
Quetin and Ross (1991) Ross et al. (1987)	– 6′ diam tank – winter-over	Flow-through	Ambient	Ambient	In situ from flow through	15 mo
Hamner and Hamner (2000)	6 diam tank, schooling	Flow-through	Ambient	Ambient	In situ from flow through	5 mo
Nicol et al. (1992); Nicol (2000)	Modification 250 ml perforated jars in 1000-L	Flow through tank		Flowing seawater	No supplement	5 d
Cuzin-Roudy, Pers commun	– 1 female plus males in 2-L – group in large tank	– floating in 200-L tank – Daily change seawater		Cold lab 0°C	'brown ice'	1 mo

~7-L of natural seawater kept cool in 6″ of running seawater, and changed water in the buckets weekly (Table I). Over half of the individuals survived and continued to molt for over 4 months under these conditions. Clarke continued to work with live Antarctic krill at intervals (Clarke and Morris, 1983; Clarke *et al*., 1988) through the next two decades.

By the early 1970s Discovery House was not the only marine laboratory in the Antarctic. The National Science Foundation of the United States built Palmer Station on Anvers Island, halfway down the Antarctic Peninsula. Work began in the late 1960s, but the first krill research was not done until the 1975–1976 austral season. Dr. Mary Alice McWhinnie (Fig. 4), often called Dr. M. A., had a long-term interest in Antarctic krill. However, for the first ten years (1962–1972), her laboratory was aboard the USNS *Eltanin* during six cruises to the sub-Antarctic and Antarctic, and at McMurdo Station in the Antarctic (1974) where either conditions for long-term maintenance were nil or Antarctic krill were absent (Land, 1981). Her field seasons included one 7000-mile cruise without a single krill (Chicago Tribune, 13 February 1974).

In late November 1975, McWhinnie sailed to Palmer Station aboard the RV *Hero*, (Fig. 5) with Captain Lenie. We join Dr. M. A. in finding Captain Lenie an invaluable resource for krill research, and as she wrote ''Captain Pieter Lenie of the *Hero* contributed greatly to sonar monitoring and particularly in all sampling'' (McWhinnie *et al*., 1976). The RV *Hero* was a wooden side-trawler one-sixth the size of the USNS *Eltanin*, but until her retirement from Antarctic work in late summer 1984 she ably served

Seagoing biologist Mary Alice McWhinnie

FIGURE 4 McWhinnie in 1974. Photograph by Eugene Smith, United States Navy.

FIGURE 5 Side-trawler RV *Hero* with stabilizing sails set. Photograph by Quetin (1982).

multiple researchers in the Peninsula region. After collection with nets, krill were kept alive on board the RV *Hero* in large deep boxes attached to the port rail and with seawater flowing continuously, until their transfer to the laboratory at Palmer Station.

McWhinnie rearranged the laboratory at Palmer Station to suit krill research, including two new krill laboratories in 1977–1978 that provided the first opportunity to maintain large stocks of living krill over long periods in flowing seawater pumped from Arthur Harbor (McWhinnie and Denys, 1978) (Table I). Populations of several thousand Antarctic krill were maintained in the laboratory during the winter for the first time in 1978 and 1979 (McWhinnie *et al.*, 1979; Denys *et al.*, 1980). They were held in glass rectangular aquaria (two tanks were $3.34 \times 0.6 \times 0.6$ m, one tank $1.7 \times 0.6 \times 0.6$ m) with automatic light control. The seawater flow ranged from $300\,\mathrm{L\,h^{-1}}$ (McWhinnie and Denys, 1978) to $150–200\,\mathrm{L\,h^{-1}}$ (McWhinnie *et al.*, 1979). Such conditions allowed for investigations of the maturation cycle (Denys *et al.*, 1980) and the behavioral role of light. As previously done by Mackintosh and Clarke, krill were also maintained in individual jars to observe growth rates (Poleck and Denys, 1982) (Table I). Denys, a graduate student, was the team leader during the last season (1979–1980) for the McWhinnie team when Dr. M. A. was struck down by an illness. She died in March 1980 as the season was coming to an end. In her honor, the laboratories at Palmer Station were re-named the Mary Alice McWhinnie Biology Center during the 1980–1981 season. Other visiting krill scientists, Meyer in 1977–1978 and Antezana and Ray of Chile in 1980 had already benefited from Dr. M. A.'s additions to Palmer Station when they conducted experiments to quantify feeding behavior (Antezana *et al.*, 1982) and feeding selectivity (Meyer and El-Sayed, 1983).

Research on living krill during the late 1970s and the early 1980s was also conducted at a third marine station, the Arctowski Laboratory of the Polish National Academy of Sciences. Arctowski is located on the shore of Admiralty Bay, King George Island, the northernmost of the Shetland Islands west of the Antarctic Peninsula. Buchholz (1985) described the development of a through-flow system in the 1978–1979 season that delivered a constant phytoplankton supply to krill in individual containers (Table I). There were two systems, one with submerged perspex jars (1.1-L) with an inflow of fresh seawater, and another with 1-L jars with two outlets for regular exchange of seawater and for feeding phytoplankton to krill. Buchholz conducted growth and molting experiments on living krill in both the 1978–1979 and 1982–1983 seasons, enjoying the easy access to Antarctic krill, the football games and the hospitality of the Polish Antarctic Expeditions. Buchholz (Fig. 6) collected krill with a net from a small (~7 m) wooden boat in Admiralty Bay which was later replaced by a somewhat larger vessel. Combined ship and laboratory studies took place on the RS *Meteor* and RVIB *Polarstern* (Buchholz, 1983, 1985, 1991). Temperature adaptation experiments were again conducted at Arctowski Station in the late 1980s and early 1990s (Vetter and Buchholz, 1997).

In the first half of the 1980s, communication among researchers from different institutions and different countries began to grow, and the exchange of techniques and live animals, and collaborative visits to other stations expanded. Boyd *et al.*

FIGURE 6 Arctowski Station's first small boat "Dzunia" used to collect krill in Admiralty Bay in 1978–1979. Buchholz is sitting; with the krill net to his left and the towing winch in the foreground. Photograph courtesy of Buchholz.

(1984), from Canada, benefited from the facilities at Arctowski Laboratory in the 1981–1982 season, studying feeding behavior of live krill, especially size selection of phytoplankton. These krill were collected by the RV *Hero* and delivered to Boyd at Arctowski when two other research groups new to the Antarctic (Hamner *et al.*, 1983; Ross and Quetin, 1983) visited Arctowski with live krill in the tanks before their return to Palmer Station. At South Georgia, Clarke introduced Morris to the laboratory facilities of Discovery House and the ease of dip netting krill from the jetty (Morris *et al.*, 1983). Then in the 1980–1981 season Morris (1984) measured filtration rates in a flow-through system of 1-L volume compared to constant volume experiments in a 2-L beaker, suggesting previous experiments had significantly underestimated filtration rates. Morris also tested a large flow-through aquarium, a prototype of that of Buchholz, and in 1982 used it in a preliminary study of the time course of the molt cycle and growth in live krill held in the aquarium at King Edward Point, South Georgia (Morris and Keck, 1984). The actual main experiment, planned by Morris and Buchholz for the following year, was prevented by the Falkland war – and the krill tanks ended up as barricades, filled with sand.

Several groups from other countries also conducted research at Palmer Station during this period. Clarke joined Quetin and Ross at Palmer Station during the 1984–1985 season and estimated body rations from fecal pellet production rates (Clarke *et al.*, 1988). Boyd headed a Canadian group in 1985–1986 that established that grazing rates were higher in experimental containers 10 times the volume of anything previously used (Price *et al.*, 1988), an important contribution to our understanding of energetics.

Conditions on board the early research vessels such as the *Discovery II* were dignified, with a drink before dinner and a small cup of coffee after dinner (Deacon, 1977). The laboratory facilities on *Discovery II*, a 234-foot steel vessel, were primitive, however, and not adequate for experimentation or observations of natural behavior of living krill or for measurements of physiological rates of freshly collected krill. Increasing sophistication in techniques of on-board maintenance in the late 1970s meant that experiments could be conducted at sea. In the 1977–1978 and 1980–1981 seasons Kils (1982, 1983) towed a camera for *in situ* observations of behavior, conducted shipboard experiments and observations in 63-L plexiglass tanks, and observed tethered krill in a flow tank or free-swimming krill in a water-ring flow chamber (Kils, 1983). See http://www.ecoscope.com/krill for Kils's krill cybermicroscope. Feeding experiments were conducted with krill held in 500 ml of natural phytoplankton (1977–1978, Kato *et al.*, 1979) and later 2 L (1983–1984, Ishii *et al.*, 1985) in an incubator on board the TS *Umitaka-Maru III*. We learned a few years later (Price *et al.*, 1988) that these volumes led to under-estimates of ingestion rates. Spawning and egg development experiments were conducted on board both the RV *Hero* (Ross and Quetin, 1983) and the RV *Kaiyo-Maru* (Kikuno, 1981; Kikuno and Kawamura, 1983).

Two Options for Progress

At the start of the decade of the 1980s there appeared to be two distinct options for additional progress in experiments and observations with living Antarctic krill – transport living krill back to sophisticated home laboratories (Table II) or increase the sophistication of laboratories in the Antarctic and bring scientists there.

TABLE 2 Transport of live krill back to the home laboratory, and conditions for rearing experiments.

	Investigator	*Transport*	*Experiments*	*Food in lab.*	*In Lab.*
Murano *et al.* (1979)	Feb. 1978 For 30 d in cooled 10- and 20-L tanks; fed, water changed/2 d	Molting and growth	*Dunaliella tertiolecta (Dt)* and *Phaeodactylum tricornutum (Pt)*	Dark, 0.7°C 1-L Daily water change	>256 d
Ikeda and Dixon (1982 a, b)	Jan. and Feb. 1980 Insulated containers, by air to AIMS	Molting and growth	Starved vs. 2 fed controls (Tetra Marin/copepods; microalgae *Dt* and *Pt*)	Dim light, −0.5°C 1 – 5-L 80%/wk	>211 d
Ikeda (1987); Continued by Nicol from 1986 to present	Dec. 1982 By ship to Australian Antarctic Div., Tasmania	Rearing, maturation; No mating observed	*Pt* = staple, with some Antarctic diatoms and prymnesiophytes Added 1×/wk	Dark, 0°C Vol. incr. with devel. to 10-L 1×/wk	3 yr 0.047 mm d^{-1}
Marschall, unpublished data	Mar. 1984 By air to AWI	Rearing, maturation. No mating.	Antarctic diatoms, cultured, high levels	Dark, −1 to 0°C	200 d 0.17 mm d^{-1}
Quetin and Ross, for collaborators: Frank and Widder (1999); McGehee *et al.* (1998)	Apr. 1983 Feb. 1995	Eye sensitivity Acoustic target	Starved	Dark, 0°C	Up to 7 months
Hirano *et al.*, this volume	Mar. and Jun. 2000 Adults by ship or from AAD to Port of Nagoya Aquarium	Rearing, maturation from eggs released by adults.	Daily phytoplankton feeding during 12 h no flow time. *Pt* at 1.2 10^5 cells ml^{-1} plus *Pavlova lutheri with vitamins in minced clam.*	Closed-filtering system. 0.4°C. 180- to 600-L aquaria	

Transport back to home laboratory The first feasibility study of transport and long-term maintenance of living krill was done in 1978 when krill collected with a fish pump were transported by the TS *Umitaka-Maru III* back to the Museum of Fisheries Science, Tokyo University of Fisheries. They were maintained in the laboratories for over 265 d for growth and molting studies (Table II) (Murano *et al*., 1979).

Ikeda *et al*. (1980) also transported living krill to a tropical laboratory, the Australian Institute of Marine Science (AIMS) in Queensland, Australia, but by air in insulated containers. Krill collected in January and February 1980 from the RV *Kaiyo-Maru* were transported to AIMS, and used by Ikeda and Dixon (Fig. 7) in experiments on molting and growth under three food regimes (Table II) (Ikeda and Dixon, 1982a,b). In addition, Ikeda kindly loaned Quetin and Ross (1985) some of the krill he had been maintaining for use in feeding experiments during their stay at AIMS as post-doctoral researchers in 1980. (We returned eight of the nine krill after 12 months of experiments.) Ikeda subsequently transported fertilized eggs collected from gravid females collected by the *Nella Dan* in late December 1982 back to the laboratories of the Australian Antarctic Division in Tasmania, Australia. A few of these eggs hatched and matured to adults in 3 years, with maximum sizes of about 45 mm and growth averaging 0.047 mm d^{-1}, on a diet of *Phaeodactylum tricornutum* supplemented with some Antarctic diatoms and *Phaeocystis pouchetti* (Table II) (Ikeda, 1987). In the spring of 1986, Ikeda returned to Japan from Tasmania, and Nicol continued the tradition of transporting live krill back to the laboratory for various experiments. Longer-term experiments than possible on board a research vessel have been conducted in the Australian Antarctic Division laboratories, including research on condition factors in living krill held under different conditions (Shin, 2000; this volume), and rearing krill to verify the lipofuscin technique for aging krill (S. Nicol and R. Harvey, personal communication).

FIGURE 7 Ikeda (looking in the drawer) and P. Dixon (at microscope) checking Antarctic krill in a cold room at the Australian Insititute of Marine Science, early 1980s. Photograph courtesy of P. Dixon.

At a workshop organized by Clarke and Mayzaud under the auspices of BIOMASS, and held the fall of 1986 in Rimouski, Quebec, Canada, a small group of krill scientists also learned of the rearing experiment conducted by Marschall of the Alfred-Wegener-Institute (AWI) in Bremerhaven, Germany. Marschall joined Quetin at Palmer Station during the 1983–1984 season. At the end of the season Marschall transported krill larvae hatched from eggs released by gravid females held at Palmer Station across Drake Passage on the RV *Hero*, and then in containers cooled with ice as hand-carry luggage on airplane flights from Chile to Germany. The stewardesses on the flight who kept the containers filled with ice treated the developing larvae as pets (U. Kils, personal communication). The developing krill were kept in a chest freezer at 0°C, and fed high concentrations of cultured Antarctic diatoms. Marschall wrote to us in February 1985: ''To be serious now – from August the 15th to the 27th of January, the fastest growing specimens grew from a total length of approximately 18 to now 42–45 mm which means a growth rate of some 0.14 mm per day averaged over five and a half months.'' By the Rimouski meeting he reported the krill grew to 30 mm in 6 months (growth of 0.17 mm d^{-1}), and then more slowly to 50 mm in another 200 d (Table II) (Clarke, unpublished report). The inescapable conclusion was that food type and quantity were important determinants of growth and development rates. However, neither Ikeda nor Marschall observed mating in the laboratory so a full life cycle – egg to egg – had not yet been observed in the laboratory.

Living krill have also been transported live back to the laboratories of Quetin and Ross at the Marine Science Institute, University of California at Santa Barbara, California, USA, for several short-term specific experiments (Table II). Krill were placed 2 per 2-L Nalgene jar of filtered seawater, surrounded by an ice/fresh water slurry in a cooler and shipped as checked baggage (Table II). They were maintained starved under similar conditions until used either for experiments on the spectral sensitivity of the eye (Frank and Widder, 1999) or for target strength determinations (McGehee *et al*., 1998) 1–7 months later.

Increase sophistication in laboratories or on board ship in Antarctic The other option was to increase the capability of the laboratories in the Antarctic, such as at Palmer Station and/or on board a research vessel. From the early days when ships were used only for collection or simple experiments conducted in on-deck incubation systems, we have moved to sophisticated laboratories and aquarium rooms inside the research vessel. Shipboard laboratories, as described above, initially often limited one's ability to work with live euphausiids. In addition, the early research ships had limited ability to penetrate into the pack ice to work with live euphausiids from the under-ice habitat. This lack was perceived as increasingly critical as we began to understand the role of sea ice in the life cycle of krill. One of the strengths of conducting experiments and observations on board the research vessel is that the scientist is working with freshly collected krill that may best represent *in situ* conditions. In addition to penetrating the ice pack, a new collection technique was introduced – SCUBA divers began collecting krill from the under-ice habitat for on-board experiments in 1986 (Marschall, 1988) and 1987 (Ross and Quetin, 1991) (Fig. 8).

One of the early primary research vessels to penetrate deep into the ice pack was the RVIB *Polarstern*, operated by the AWI. RVIB *Polarstern* was commissioned in 1982, has had 27 expeditions to the Arctic and Antarctic, and has allowed scientists to enter the ice pack in winter. Research on living krill on the RVIB *Polarstern* has

ranged widely over the past two decades, and only a few examples are mentioned to illustrate the variety possible. Studies in the 1980s included: (1) observations of feeding behavior in the cold laboratory during the winter and early-spring in the pack ice of the Weddell Sea (Marschall, 1988), and (2) detailed investigations of krill maturation and mating in the spring and summer 1988–1989 in the Scotia-Weddell Sea (Cuzin-Roudy and LaBat, 1992) (Table I) (Fig. 9). More recently, in the Lazarev Sea in austral fall 1999, Atkinson *et al*. (2002) and Meyer *et al*. (2002) conducted experiments aimed at determining the energy budget of larval, juvenile and adult krill just prior to the critical over-wintering period.

Over the years, instantaneous growth rate (IGR) experiments, representing rates of growth *in situ* of live, freshly collected krill, have become increasingly common. Quetin and Ross (1991) developed the IGR method in 1985, and used seawater tables to cool the experimental vessels during 4-d experiments on board the MV *Polar Duke*, an ice-strengthened vessel that replaced the RV *Hero*, (Table I). Nicol *et al*. (1992) subsequently determined that laboratory conditions began to affect the growth increment after about 5 d. Nicol (2000) also briefly described a modification of the original instantaneous growth rate protocol that was used on board the RSV *Aurora Australis* in 1996, with temperature control via a cold room (Fig. 10). The new version of IGR increases the sample size for an experiment by 10 times, a significant improvement for statistical analysis (Table I).

The United States and the British Antarctic Survey followed two approaches for facilities for research on living krill – laboratories in the Antarctic and on board ice-breaking vessels. The resources are similar, with small boats operating in waters near to the marine laboratories, and large research vessels for research further afield. For BAS, two major facilities are the *James Clark Ross* (Fig. 11), and the new Bonner Laboratory at Rothera Base on Adelaide Island. On a recent voyage on the *James Clark Ross* to South Georgia in January–February 2001, Atkinson, Meyer, and Tarling conducted many experiments on living Antarctic krill, including IGR experiments. Although marine biology research at Rothera primarily focuses on the benthic environment and its relationship to the pelagial, some interesting research on krill physiology was carried out there in late summer 2000 by Meyer and Atkinson. Adult krill were brought to Rothera by the ASRV *Laurence M. Gould* (adults) during the Palmer Long-Term Ecological Research (LTER) annual cruise, and furcilia larvae were collected by hand towing nets from a small boat near the base. Unfortunately, the Bonner laboratory of Rothera burned to the ground in October 2001, but is being rebuilt.

The United States Antarctic Program also has both ship and station facilities. More recently the RVIB *Nathaniel B. Palmer* (commissioned in 1992) and the ice-strengthened ASRV *Laurence M. Gould* (commissioned in 1998) have been instrumental in supporting the U.S. portion of the Southern Ocean GLOBEC program (Hofmann *et al*., 2002), and the Palmer LTER. Experiments with living Antarctic krill were conducted in cold vans, aquarium rooms and controlled temperature laboratories in both the austral fall and winters of 2001 and 2002. Palmer Station is the USAP marine laboratory located in a region where krill can be abundant, and where we have worked since the 1981–1982 Antarctic season. Our first season (1981–1982) we enjoyed the RV *Hero* and Captain Lenie (Fig. 12). Living krill are brought to station either by one of the research vessels (RV *Hero* until austral fall 1984, MV *Polar Duke* January 1985–March 1997, ARSV *Laurence M. Gould* January 1998 to present) or collected with a ring net and hydraulic winch from a Mark V zodiac (Fig. 13).

FIGURE 8 The under-ice habitat: clockwise from top left, SCUBA diver swimming in over-rafted region, larval krill in over-rafted area, divers at surface, diver collecting Antarctic krill. Photographs by Quetin and D. Martin from 1991 to 1999.

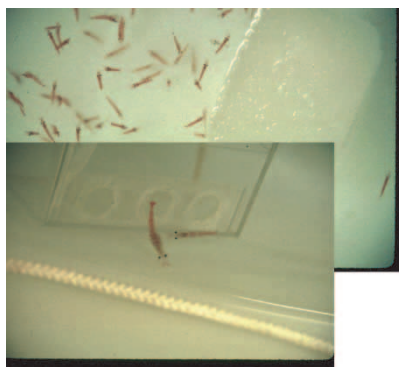

FIGURE 9 Maintenance and experimental setups in 1988–1989 inside RVIB *Polarstern*. Juvenile krill maintained at 0°C in a large tank of seawater. Krill were fed with ice algae released from a block of "brown ice," and water renewed once a day. Gravid female and male Antarctic krill were paired for mating and isolated in small boxes inside a larger tank with other krill. Photographs courtesy of Cuzin-Roudy.

FIGURE 10 Modified IGR setup inside *Aurora australis* in 1996 with R. King seated to check the krill. Photograph courtesy of Nicol.

FIGURE 11 *James Clark Ross* in ice during the 2001 cruise near South Georgia. Photograph courtesy of G. Tarling.

FIGURE 12 Ross, Captain P. Lenie and Quetin on bridge of RV *Hero* at the end of the 1981–1982 season.

FIGURE 13 Mark V zodiac, ''Rubber Duke II,'' trawling in the waters near Palmer Station during the austral season 2000–2001. Photograph courtesy of Palmer Station personnel working for the Palmer Long-Term Ecological Research Project.

FIGURE 14 Views of Palmer Station, operated by the National Science Foundation, USA. Approaching Palmer Station. Photograph by Quetin and Ross.

FIGURE 15 Rolling the new 2-m tanks for maintaining krill and fish up the walkway to the aquarium building in 1986. Photograph by Quetin and Ross.

Palmer Station (Fig. 14) went through several renovations, beginning with those of McWhinnie. In the mid-1980s, the laboratories at Palmer Station were updated in order to conduct both physiological and behavioral experiments on living krill under controlled conditions. The small krill laboratories with rectangular tanks in a flowing seawater system were replaced by a large aquarium building with two controlled temperature rooms. A flowing seawater system was plumbed into two cascade tanks and four 2-m diameter round tanks about 1.5 m in height (Figs. 15 and 16). These round tanks proved ideal for keeping krill for long periods of time to observe maturation under ambient conditions (Ross *et al*., 1987), and to observe schooling and feeding on melting ice blocks (Stretch *et al*., 1988; Hamner and Hamner 2000). Mating was observed in the austral spring in a group of krill that winter-overed in one of

FIGURE 16 K. Haberman cleaning out the 2-m tanks inside the aquarium building in 1992. Photograph by Quetin and Ross.

the tanks, and several of the females subsequently released fertilized eggs. However, we did not attempt to rear those hatched larvae to adult. Hamner and Hamner (2000) discovered that schooling behavior could be generated if care was taken in setting up the environmental conditions. Dim light with no sharp contrasts appeared to be best – and a white towel over Hamner's dark hair and beard improved the schooling response significantly (Hamner, personal communication).

The environmental rooms have fostered winter-long experiments on larval growth and development under different food, temperature and photoperiod conditions (Elias, 1990), and on stable isotope turnover rates (Frazer *et al*., 1997), and short-term experiments on feeding selectivity (Haberman, *et al*., 2003a,b). Such controlled experiments would not have been possible with the original facilities. The flowing seawater tables in the cascade tanks have allowed IGR experiments with krill collected near the station to be conducted throughout an entire spring–summer season of 6-months in order to test possible correlations between growth and *in situ* environmental conditions (Ross *et al*., 2000). This set of experiments required both full-season access to living krill in the environment, and nearby laboratory facilities to conduct the experiments.

Continuing Advances in Rearing and Maintenance

The most recent breakthrough is a result of the research-scientists at the Port of Nagoya Aquarium who developed a technique to maintain krill in large numbers with a recycling filtration system (Hirano and Matsuda, 2003) (Table II). With this rearing method, Antarctic krill have reproduced in captivity, and gone through several generations in the laboratory – a first in the long history of working with living krill. With the

establishment of captive populations, it is now possible to conduct experiments such as those reported in this volume, i.e. photoperiod and its relation to the maturity and spawning of Antarctic krill and larval development (Hirano *et al*., 2003; Yoshida, accepted).

At this time there are several laboratories that are poised to conduct extensive research on populations of living krill – the Australian Antarctic Division, the Port of Nagoya Aquarium and Palmer Station, Antarctica. Many of the current research vessels are well equipped with cold laboratories or aquarium rooms with flowing seawater to facilitate experiments ranging from hours to days to the duration of the cruise. As our understanding of the best conditions for maintenance and rearing has grown over the 75 years since Mackintosh first kept Antarctic krill alive, we have learned many things about optimal conditions for Antarctic krill. Light, temperature, the volume of the vessel and the quantity and quality of the food available are the important factors affecting the results of experiments on both physiological rates and rearing, as well as behavioral observations. Future experiments on living krill will build on the results of the past illustrious lineage of krill scientists.

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