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The effects of daily fluctuating temperatures and dimethylnaphthalene contaminated food on the estuarine grass shrimp, palaemonetes pugio

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THE EFFECTS OF DAILY FLUCTUATING TEMPERATURES AND DIMETHYLNAPHTHALENE-CONTAMINATED FOOD ON THE ESTUARINE GRASS SHRIMP, PALAEMONETES PUGIO

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TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (concluded)

ABSTRACT

The physiological effects of a natural perturbation (fluctuating temperatures) and a petroleum-induced perturbation (dimethylnaphthalene-contaminated food) on the grass shrimp Palaemonetes pugio were compared. The resistance of shrimp to environmental challenge, oxygen consumption rates ($\dot{V}O_2$) and several physiological indices of stress were determined after a 32 day exposure to fluctuating temperatures (FT) (18-22°C) and/or dimethylnaphthalene (DMN)-comtaminated food (0.24 µg DMN/g wet wt) and again after a 16 day recovery period of stable temperatures (20°C) and uncontaminated food.

Both FT and DMN-contaminated food reduced survival to the challenge of hypoxia + reduced salinity. FT were quantitatively more stressful than either stable temperatures or DMN-contaminated food. After the recovery period, FT did not affect survival to hypoxia, while shrimp, which had ingested DMN-contaminated food, exhibited enhanced survival to hypoxia.

Ingestion of DMN-contaminated food for 32 days resulted in elevated $\dot{V}O_2$ in shrimp exposed to declining oxygen concentrations. After the 32 day exposure period, FT had no significant effect on $\dot{V}O_2$ at 15°C, 20°C and 25°C, tissue $\dot{V}O_2$, $\dot{V}O_2$ in declining oxygen and hemolymph copper concentrations. After the 16 day recovery period, shrimp from the FT regime exhibited depressed $\dot{V}O_2$ when exposed to 25°C but not to 15°C. These depressed respiratory rates were offset by the stimulatory effect of DMN-contaminated food.

The ratio of oxygen consumed to nitrogen excreted was elevated after the exposure and recovery periods in shrimp exposed to both FT and DMN-contaminated food at the same time. Water flux rates were elevated by FT after the exposure and recovery periods but not when DMN-contaminated food was also ingested. Both water flux rates and the ratio of oxygen consumed to nitrogen excreted were elevated in all shrimp after the recovery period relative to levels observed after the exposure period . After the exposure period, FT induced elevated hemolymph acid phosphatase activities in shrimp exposed to hypoxia. After the recovery period, hypoxia induced elevated hemolymph acid phosphatase activities in shrimp which had ingested nothing but uncontaminated food relative to those ingesting DMN-contaminated food.

THE EFFECTS OF DAILY FLUCTUATING TEMPERATURES AND DIMETHYLNAPHTHALENE-CONTAMINATED FOOD ON THE ESTUARINE GRASS SHRIMP, *PALAEMONETES PUGIO*

INTRODUCTION

Seventy-five percent of all oil entering the aquatic environment is released into the coastal zone. The majority of this input is in the form of slow, chronic releases which generally occur undetected (Farrington, 1975; National Academy of Sciences, 1975). This input of petroleum, along with human population density, is expected to increase in coastal regions (Ketchum, 1972; Sittig, 1978). Concomitant with this increase is a requirement for better ways to assess the health of the biota inhabiting this highly productive area.

In the last 15 years much research has attempted to assess the effects of oil on marine and estuarine ecosystems. However, in many of these studies, the natural variation in populations and community structure has often been of such magnitude that detecting any change due to the presence of oil has been very difficult or impossible (Bowman, 1978; Cowell, 1978; MacIntyre et al., 1978; Moore and McLaughlin, 1978; Myers, Southgate and Cross, 1980; Dillon and Lynch, 1981). Since changes in populations and community structures are predicted on the fact that a change in the competitive fitness of individual organisms has occurred, some workers have suggested evaluating stress in the individual organism. This approach could possibly detect stressed populations during the early stages of the perturbation. Jeffries (1964; 1972) attempted such an approach by measuring physiological and biochemical parameters in marine organisms

and found that natural variations in these indices made clear interpretation of the results very difficult.

Lynch (1974) followed a similar approach and concluded that after accounting for natural variations in several biochemical and physiological parameters, stress in the blue crab Callinectes sapidus could be identified by the use of these indices. In a later paper, Dillon and Lynch (1981) reviewed the feasibility of using physiological indices to detect stress in marine and estuarine organisms and concluded that although this approach did hold strong potential, there was a paucity of data directly comparing stressed induced by exposure to a pollutant such as oil and stress due to natural variations in the environment. This investigation attempts to compare the biological effects on an estuarine organism of a natural perturbation, in the form of daily fluctuating temperatures, with those resulting from the chronic ingestion of oil-contaminated food.

Fluctuating temperatures (FT) may represent a real stress for animals in the estuarine environment since most are poikilothermic. There are conflicting reports as to whether daily variations in temperature increase (Hubbs 1964; Lindsey and Ali, 1965) or decrease (Sylvester, 1974; Hokanson, Kleiner and Thorslund, 1977) survival in fish. Brett (1971) has demonstrated the energetic advantage of FT in vertically migrating salmon. High feeding rates at shallow depths (higher temperatures) coupled with high conversion efficiencies in deep water (lower temperatures) resulted in more energy being available for growth. McLaren (1963) has demonstrated a similar phenomenon in vertically migrating zooplankton.

Studies on the effects of FT on marine invertebrates have been primarily limited to two groups, molluscs and decapod crustaceans. FT has little effect on the survival, shell formation or organic carbon content of Crepidula fornicata except when the FT regime entailed exposure to very high stressful temperatures (Lucas and Costlow, 1979). A significant increase in the resistance to elevated temperatures has been reported for gill-epithelium of the bivalve mollusc, Mytilus edulis, exposed to FT for only 4 days (Huppert and Laudien, 1980). Widdows (1976) also reported increased thermal tolerance for Mytilus edulis exposed to FT, an increase in the thermal independence of aerobic metabolism and an overall increase in the mussel's scope for growth (i.e., energy available for activities other than maintenance).

The effects of FT on both larval and adult decapod crustacea have been examined. FT confer a slight (Costlow and Bookhout, 1971; Rosenberg and Costlow, 1976) to highly significant stimulatory effect on the survival of larval crabs (Sastry, 1976). FT increased respiration rates in the larvae of Cancer irroratus exposed to abrupt temperature increases but not decreases (Sastry, 1979). Survival of larval Crangon septemspinosa maintained in FT was intermediate to that of larvae held at stable temperatures equivalent to the extremes of the FT regime (Regnault and Costlow, 1970). Adult crabs, Panopeus herbstii and Uca pugilator, exhibit a general reduction in oxygen consumption rates after experiencing FT for 15 to 20 days (Dame and Vernberg, 1978). Growth was unaffected by FT in adult crayfish, Procamborus acutus acutus although mortality was higher than for

crayfish at stable temperatures (Thorp and Wineriter, 1981). Except at low salinities, FT had no significant effect on survival of two sympatric species of adult grass shrimp, Palaemonetes pugio and P. vulgaris (Thorp and Hoss, 1975). These authors also reported no effects of FT on the oxygen consumption rates of shrimp transferred to low temperatures. There appears to be no consistent response of marine invertebrates to FT. This may be due, in part, to widely varying methodologies as well as to interspecific and developmental differences.

A considerable amount of information has been published concerning the lethal and sublethal effects of oil in seawater on marine and estuarine organisms (Anderson, 1979). Much of this work has shown that the aromatic fraction of oil is primarily responsible for the observed toxicity and sublethal effects of oil. Data gathered under actual spill conditions in the field have shown that the concentrations of aromatic hydrocarbons in the water column are generally low, often one to several orders of magnitude below sediment concentrations (Cox, Anderson and Parker, 1975; Armstrong et al., 1979; Bieri, Stamoudis and Cueman, 1979).

The uptake of aromatic hydrocarbons from seawater is generally rapid in most marine organisms. The release or depuration of these compounds is also rapid once these organisms are placed in oil-free seawater (Neff et al., 1976; Anderson, 1979). By contrast, the uptake of aromatic hydrocarbons from heavily contaminated sediments is generally slow, with most of the uptake believed to be due to hydrocarbons in the interstitial pore waters (Anderson, Kiesser and

Blaylock, 1979). Despite this apparently low bioavailability, oil-contaminated sediments can have a significant impact on mortality and community structure (Armstrong et al., 1979; Fletcher, Kiceniuk and Williams, 1981). It is still unclear to what extent oil-contaminated water and oil-contaminated sediments contribute to tissue concentrations of aromatic hydrocarbons found in organisms taken from oil-polluted environments.

The chronic ingestion of oil-contaminated food by aquatic organisms has received little attention relative to contamination via oiled seawater or sediments. Singer et al. (1980) suggested that the uptake of aromatic hydrocarbons through the diet may be a major route of contamination because of their lipid solubility. Neff (1979), in summarizing the importance of oil-contaminated food on aquatic crustaceans, concluded that although they did rapidly assimilate petroleum via the diet, it was eventually depurated from the tissues. All of the work which Neff (1979) reviewed utilized a single dose of contaminated food. At present, there is no comparable information which would indicate what effects the long-term ingestion of oil-contaminated food by estuarine crustaceans might be.

As pointed out before, there is a body of evidence which indicates that the effects of whole oil, on a variety of marine organisms, is dependent on the relative size of the aromatic fraction, especially the diaromatic naphthalenes (Anderson et al., 1974a; b). Laboratory studies with aquatic crustaceans have shown that dietary naphthalene is taken up more efficiently, is metabolized more slowly and is depurated from the tissues at a slower rate than naphthalene in

solution (Corner et al., 1976; Harris et al., 1977). Dimethylnaphthalene (DMN), an alkylated homolog of naphthalene, is more toxic than naphthalene to many marine organisms and is persistent in oil-contaminated environments and organisms (Anderson et al., 1974b; Cox et al., 1975; Tatem, 1975; Gordon et al., 1976; Bieri et al., 1977; Armstrong et al., 1979). For these reasons, oil-contaminated food in this study is represented by a DMN-contaminated food source.

The estuarine shrimp Palaemonetes pugio was utilized in the present study for several reasons. It is an abundant and cosmopolitan member of the estuarine community and serves a vital role in the flow of energy by ingesting meiofaunal organisms (Bell and Coull, 1978; Nelson, 1979; Morgan, 1980) and particulate organic matter, including fecal pellets (Johannes and Satomi, 1966; Adams and Angelovic, 1970; Nixon and Oviatt, 1973; Welsh, 1975). Palaemonetes pugio, in turn, serves as food to consumers at several different trophic levels (Darnell, 1958; Wood, 1967; Nixon and Oviatt, 1973; Stickney, Taylor and White, 1975). The ingestion of particulate matter and fecal pellets by Palaemonetes pugio may be significant with respect to the potential for contamination by oil since these two represent major routes of vertical transport of aromatic hydrocarbons from the water column to the benthic environment below (Conover, 1971; Lee et al., 1978; Prahl and Carpenter, 1979). Palaemonetes pugio is a eurytolerant organism which exhibits a high degree of regulation of both oxygen consumption rates and the osmotic pressure of its body

fluids (McFarland and Pickens 1965; Thorp and Hoss, 1975; Roesijadi et al., 1976a; b).

There is a considerable amount of information available regarding the lethal and sublethal responses of Palaemonetes pugio to whole oil, water-soluble fractions of whole oil and to specific aromatic hydrocarbons, including DMN. Anderson et al. (1974a) performed bioassays on six estuarine species exposed to seawater soluble fractions and dispersions of four types of oils and found Palaemonetes pugio to be the second most sensitive organism. Similarly, Hall, Buikema and Cairns (1978a) reported Palaemonetes pugio to be more sensitive to an artificial refinery effluent containing No. 2 fuel oil than the pinfish Lagodon rhombroides. The 96 h LC_{50}s (lethal concentrations causing 50% mortality after 96h) for Palaemonetes pugio exposed to naphthalene, methylnaphthalene and DMN were 2.35, 1.1 and 0.7 mg/l, respectively (Tatem, Cox and Anderson, 1978).

Tatem (1977) reported that Palaemonetes pugio rapidly accumulated DMN from seawater solutions of oil with tissue concentrations being 150 times greater than water concentrations of 0.1 mg/l after only 6 h. Depuration began while shrimp were still in the exposure solution but was most rapid 24 h after transfer to oil-free seawater. However, a low residual concentration (0.1-0.2 ug/g) remained in shrimp tissue even after 21 days in clean seawater. During the depuration period DMN remained in the exposure water and in the shrimp tissues to a greater extent than any of the other naphthalene homologs. Exposure of Palaemonetes pugio to oil in seawater has been reported to have an inhibitory effect on oxygen consumption, egg

production, larval hatching, growth and survival (Tatem, 1977; Buikema, Niederlehner and Cairns, 1980).

Mammals generally respond to stress in a consistent manner (Selye, 1955). This response is characterized by the stimulation of the hypothalamus-pituitary-adrenal axis to produce altered blood concentrations of corticosteroid hormones. Three distinct response phases consisting of an alarm reaction, a resistance stage and adaptational stage are usually observed. The ultimate objective in the mammalian response to stress is the maintenance of a constant internal steady-state, or homeostasis. Some, but not all, non-mammalian vertebrates exhibit a similar response to stress (Donaldson and Dye, 1975; Siegel, 1980; Thomas, Woodin and Neff, 1980; Peakall et al., 1981).

An analogous, consistent response to non-specific stress is not observed in aquatic invertebrates, although they may respond to environmental change in a triphasic fashion (Kinne, 1967). The lack of a uniform response to stress is due in part to the lack of an hypothalamus-pituitary-adrenal axis. However, it is primarily the result of an entirely different strategy used to cope with environmental change. Rather than maintain a constant internal steady-state, they adapt enantiostatically, i.e., an alteration in one internal parameter induced by environmental change is opposed by adjustments in one or more other internal parameters resulting in physiological stability but not necessarily homeostasis (Mangum and Towle, 1977). Since deviations from steady-state cannot be used to

identify stressed invertebrates, a definition of stress in these organisms has been difficult. Bayne (1975) has attempted to resolve this dilemma by defining stress in marine invertebrates to be,

> "a measurable alteration of a physiological (or behavioral, or biochemical, or cytological) steady-state which is induced by an environmental change, and which renders the individual (or the population, or the community) more vulnerable to further environmental change."

Many workers have attempted to identify certain biochemical and physiological parameters as indices of stress in marine organisms (Jeffries, 1972; Thomas, 1972; Lynch, 1974; Wedemeyer and Yasutake, 1977; MacIntyre et al., 1978; National Academy of Science, 1980; Dillon and Lynch, 1981). The value of physiological indices of stress lies in the fact that they may be early warning signs, signaling a possible detrimental effect prior to changes in the population and community structure and function. Several parameters will be evaluated in this study as potential indices of physiological stress in Palaemonetes pugio. These are: the ratio of oxygen consumed to nitrogen excreted, apparent water permeability and the level of non-specific acid phosphatase activity in the hemolymph of grass shrimp.

The atomic ratio of oxygen consumed to nitrogen excreted (O:N) is a measure of the relative reliance of an organism on protein reserves to meet its energy requirements. The O:N ratio decreases as more

protein is catabolized because less oxygen is required to oxidize proteins, relative to carbohydrates and lipids, and because the amount of nitrogen excreted as ammonia and/or amino acids increases. O:N ratios for unstressed marine crustaceans normally range from 25-40 (Snow and Williams, 1971; Conover and Mayzaud, 1975; Capuzzo and Lancaster, 1979; Clifford and Brick, 1979). Depressed O:N ratio have been reported for marine organisms stressed by low food rations, reproductive activity, and changes in temperature and salinity (Ansell and Sivadas, 1973; Bayne, 1975; Mayzaud, 1976; Capuzzo and Lancaster, 1979; Quetin, Ross and Uchio, 1980). Snow and Williams (1971) determined mean O:N ratios for Palaemonetes varians to be 34.2 in summer and 6.1 in winter, with ammonia being the major excretory product. Similar seasonal variations in O:N ratios have been reported for other marine organisms and were shown to be depressed in winter when food supplies were low and protein catabolism high (Bayne, 1973; Conover and Mayzaud, 1975). The use of O:N ratios as an accurate indicator of physiological stress has been suggested by Widdows (1978).

The cycling of water through estuarine organisms, measured via radiolabeled water, has been referred to as apparent water permeability (Smith, 1976; Dall and Smith, 1978; Cornell, 1979; Bolt et al., 1980). The term "apparent" is entirely appropriate since factors other than altered membrane permeability may be affecting water flux rates. These factors may include changes in drinking rates, branchial chamber irrigation, cardiac output and systemic circulation (Tucker and Harison, 1974; Cornell, 1979; Loretz, 1979).

However, Cantelmo (1977) has shown that the water permeability of isolated tissues from various crustaceans mirrors water flux rates in the whole animal. Most studies on water flux in aquatic crustaceans have been primarily concerned with the effects of salinity. They have shown that crustaceans can rapidly alter water flux rates when placed in water of a different salinity (Capen, 1972; Lockwood, Inman and Courtenay, 1973; Roesijadi et al., 1976a) and that euryhaline crustaceans generally have lower flux rates than stenohaline forms (Smith, 1970; Subramanian, 1975; Cornell, 1979; Bolt et al., 1980). In Palaemonetes pugio, water flux rates are diminished to a similar degree when shrimp are exposed to toxic polychlorinated biphenyls or to abrupt changes in salinity (Roesijadi et al., 1976a). This led the authors to suggest that diminished water flux rates were a generalized stress response in Palaemonetes pugio.

Lysosomes are cellular organelles containing hydrolytic enzymes capable of degrading all major classes of macromolecules. Their two primary functions are intracellular digestion of food and the controlled degradation of cellular material during development and tissue differentiation (Dingle and Fell, 1969a; b; Dingle, 1973). Disruption of normal lysosome function is often associated with tissue necrosis and pathological conditions (Dingle and Fell, 1969b; Gelman et al., 1981; Poole and Mort, 1981). A variety of substances are known to destabilize lysosomal membranes resulting in the uncontrolled release of hydrolytic enzymes (Koenig, 1969; Slater, 1969). Destabilization of lysosomes has been suggested as the mode of toxicity in organisms exposed to heavy metals. (Das and Banerjee,

1980; Roesijadi, 1980; Young et al., 1981), chlorinated hydrocarbons (Rogers, Mellor and Safe, 1976; Sastry and Sharma, 1978) and petroleum hydrocarbons (Allison and Dingle, 1966; Zbytniewski et al., 1978; Moore, 1979). The relative stability of the lysosome membrane and subsequent release of hydrolytic enzymes has been suggested as an index of physiological stress in marine organisms (Bayne et al., 1976). Acid phosphatase, a lysosomal enzyme which hydrolyzes phosphate ester bonds, has been used as a marker enzyme for increased lysosome activity (Chan and Saleuddin, 1974; Monin and Rangneker, 1974; Pomponi, 1979; Brandenburger and Eakin, 1980; Gallis, Belloc and Beauvie, 1980).

The objectives of this study were threefold: 1) to compare the effects of daily fluctuating temperatures with those of dimethylnaphthalene-contaminated food, 2) to determine the mechanism(s) which may be most affected by each of these perturbations, and 3) to identify a parameter or series of parameters which may be useful in discriminating between effects due to fluctuating temperatures and those resulting from DMN-contaminated food.

MATERIALS AND METHODS

Grass shrimp were collected by dip net in the York River near Gloucester Point, Virginia. Shrimp were identified as Palaemonetes pugio according to Holthuis (1952). They were maintained in 17 °/oo salinity York River water at 22°-23°C for 1-2 days before use. For all experiments, artificial seawater prepared with sea salts (Aquarium Systems, Mentor, Ohio) diluted with either distilled water for 2 °/oo or with well water for 17 °/oo was used. Salinities were determined with a hand-held refractometer (American Optical, Buffalo, New York).

Dimethylnaphthalene-Contaminated Food

A dimethylnaphthalene (DMN)-contaminated food source was prepared by exposing freshly hatched Artemia sp. (San Francisco Bay brand) nauplii to 2,6 DMN (Chemical Samples Co., Columbus, Ohio) at a concentration of 266 $\pm$ 7.72 µg/ℓ (n=3). After 8 h exposure, Artemia sp. were removed with fine Nitex screen, rinsed in 17 °/oo, drained and frozen in trays partitioned into 10 mm cubes. The resultant food cubes contained 0.24 $\pm$ 0.06 µg DMN/g wet wt (n=9). This concentration is approximately one-third the concentration of DMN in seawater which is acutely toxic to Palaemonetes pugio (Tatem et al., 1978). Artemia sp. prepared in a similar manner, but without DMN exposure, served as food for shrimp receiving an uncontaminated food source. All food cubes were kept frozen until needed.

To help quantify the feeding regime, the caloric content of Artemia sp. nauplii and the daily caloric requirement of Palaemonetes pugio were calculated to be 330 calories/food cube (110 mg wet wt) and 7.83 calories/shrimp/d, respectively based on caloric data reported by Anderson (1977). One food cube is sufficient to supply the caloric requirements of 40 shrimp. This ration was doubled to help eliminate effects of food deficiency.

General Experimental Protcol

Shrimp were exposed for 32 days in four aquaria (110 shrimp/aquarium) to the four experimental treatments of: stable temperature (20°C) and clean food (ST/CLEAN); stable temperature (20°C) and DMN-contaminated food (ST/DMN); fluctuating temperature (18°-22°C) and clean food (FT/CLEAN); fluctuating temperature (18°-22°C) and DMN-contaminated food (FT/DMN). The 32 day exposure was selected as an appropriate period because it is much longer than the time normally allowed in acute exposure studies. In addiiton, this period of time tends to minimize complications such as reproductive activity. A daily fluctuation of 4°C about a mean of 20°C is representative of bottom temperature variations occurring in the environment in which these shrimp were collected (Moore, 1974; Mangum, 1976). All four aquaria were immersed in a refrigerated (10°C), recirculating water bath. Mercury contact thermoregulators, coupled with 1000 watt Vicor heaters, provided accurate (± 0.2°C) temperature control. In the FT regimes, a rise in temperature was induced at 0600 by a 24 h timer switch which activated a

thermoregulator set at 22°C. At 1800, this circuit opened, allowing the seawater to cool passively until another thermoregulator began to regulate at 18°C. This resulted in a temperature cycle of approximately 12 h at 22°C and 12 h at 18°C with heating and cooling times of 25 and 40 minutes, respectively. Similar rates of temperature change have been utilized by other workers investigating the effects of FT on estuarine invertebrates (Widdows, 1976; Dame and Vernberg, 1978; Sastry, 1979; Huppert and Laudien, 1980; Thorp and Wineriter, 1981) and have been recorded in shallow water embayments similar to those inhabited by Palaemonetes pugio (Widdows, 1976; Dale and Gillespie, 1977; Smith, 1977). Clean or contaminated food was provided on a daily basis at about 1200. Each of the four treatment aquaria contained a charcoal corner filter and 37 ℓ of 17 °/oo seawater. At this salinity Palaemonetes pugio is isosmotic with the external medium (Roesijadi et al., 1976a). New filters and seawater were provided midway (16 day) through the 32 day exposure period. After the exposure period, a number of the shrimp were transferred to aquaria each containing a charcoal filter and 37 ℓ of new 17 °/oo seawater. They were given equal daily rations of uncontaminated Artemia sp. food cubes and maintained at a constant temperature of 20°C. This recovery period was designed to evaluate the persistence of the four experimental treatments. All lethal and sublethal measurements were performed both after the 32 day exposure period and after the 16 day recovery period.

Survival to Environmental Challenge

The effects of FT and/or DMN-contaminated food on the ability of Palaemonetes pugio to survive various combinations of environmental

extremes were evaluated. The actual test conditions and the challenges they represent are shown in Table 1. The level of challenge used in these tests represent temperatures, salinities, and dissolved oxygen concentrations found in the environment in which these shrimp live (Moore, 1974). The response to simultaneous changes in several parameters was utilized to approximate more closely conditions found in the field. Fifteen to 18 shrimp were transferred from each of the four experimental treatments into each of the challenges. Test chambers were 1 gal glass rectangular aquaria containing 3 ℓ of seawater. Each was immersed in a water bath of the appropriate temperature. Hypoxic conditions were created by bubbling prepurified nitrogen gas through individually controlled airstones. Oxygen concentrations were maintained at 2.0 $\pm$ 0.5 mg oxygen/ℓ and were monitored at each observation or at least every 12 h with a dissolved oxygen probe and meter (Yellow Springs Instrument Co.). Constant aeration was provided in the non-hypoxic test chambers. Observations of mortality were made after: 1, 2, 3, 4, 5, 6, 7, 8, 16, 20, 24, 28, 32, 42, 48, 56, 66, 72 h and once every 24 h thereafter until 95-100% mortality occurred. The estimated time to 50% mortality (ET_{50}) was calculated by the method of Litchfield (1949). Survival among the different challenge tests were compared with analysis of variance and subsequent comparison of the means was performed according to Steele and Torrie (1960). Some ET_{50} data within each challenge test were statistically compared by the method of Litchfield (1949).

Table 1. Summary of challenge tests after the exposure and recovery periods showing actual test conditions. 15-18 shrimp/challenge test.

Challenge Test	Challenge of	Actual Conditions		
		Temperature	Oxygen	Salinity
	After Exposure			
1	Elevated temperature + hypoxia + reduced salinity	33°C	2 mgO_2/ℓ	2 °/oo
2	Elevated temperature + hypoxia	33°C	2 mgO_2/ℓ	17 °/oo
3	Elevated temperature + reduced salinity	33°C	7 mgO_2/ℓ	2 °/oo
4	Hypoxia + reduced salinity	20°C	2 mgO_2/ℓ	2 °/oo
	After Recovery			
1	Hypoxia + reduced salinity	20°C	2 mgO_2/ℓ	2 °/oo
2	Hypoxia	20°C	2 mgO_2/ℓ	17 °/oo
3	Reduced salinity	20°C	9 mgO_2/ℓ	2 °/oo

Oxygen Consumption Studies

The sublethal effects of FT and/or DMN-contaminated food on oxygen consumption rates ($\dot{V}O_2$) of 6-12 shrimp/treatment were determined at 15°, 20° and 25°C with a Gilson differential respirometer following the general procedures and calculations of Dunn and Arditti (1969). The shaking motor was adjusted to produce 130 oscillations/min. Each respiratory chamber contained 5 ml 17 °/oo seawater and 0.2 ml 20% KOH in the sidearm as a CO_2 absorbent. After 15-20 min equilibrium, the valves were closed and measurements begun. After 1.5-2.5 h, shrimp were removed to tared aluminum sheets and frozen. They were subsequently thawed, dried to a constant weight at 60°C and weighed. Values of $\dot{V}O_2$ are expressed as μlO_2 consumed/mg dry wt/h. Values were not calculated for shrimp which had molted in the respiratory chambers or had come in contact with the CO_2 absorbent.

In addition to whole animal respirometry, $\dot{V}O_2$ of dissected shrimp tissue (10 shrimp/treatment) were determined on the Gilson respirometer at 20°C. Shrimp were cut in two pieces between the tail and cephalothorax and both halves placed in the respirometry chambers. After 2 h of measurements, tissue was still quite viable as indicated by considerable movement of both shrimp halves. Preliminary experiments indicated that shrimp dissected into quarters resulted in tissues which did not remain viable but which became opaque and exhibited no viable motion after respirometry.

The influence of declining oxygen concentrations on $\dot{V}O_2$ at 20°C was evaluated in closed respirometer chambers. Two shrimp from each

of the four treatments were placed in beakers containing 100 ml of 17 $^{o}/_{oo}$ seawater. The beakers were sealed with rubber stoppers each containing a dissolved oxygen probe (Yellow Springs Instrument Co.). Declining oxygen concentrations were recorded every 10 min for 3 h. One minute prior to each observation, magnetic stirrers were turned on to rotate stir bars at the bottom of each beaker. These stir bars were separated from shrimp with a fine Nitex mesh. Constant illumination and temperature were provided by placing all apparatus, except the dissolved oxygen meter, in an incubator (Model 818, Precision Scientific Group). After each test, shrimp were placed on tared aluminum sheets, frozen and later dried to constant weight at 60°C. $\dot{V}o_2$ of shrimp was plotted against oxygen concentrations expressed as a percentage of the initial reading. There were 3 and 6 replicate tests for each treatment before and after the recovery period, respectively.

The concentration of copper in the hemolymph of 6-12 shrimp/ treatment was determined before and after the recovery period. Hemolymph copper is an accurate indicator of the respiratory pigment hemocyanin in decapod crustaceans (Djangmah and Grove, 1970; Martin, Wormhoudt and Ceccaldi, 1977). After blotting the carapace dry, the pericardium was entered thru the articulating membrane dorsal to the pericardium with a drawn-out disposable pipette. Hemolymph was deposited on a clean concave slide. From this, a 10 µl subsample was taken, prior to clotting, with microcapillary tubes (Drummond Scientific Co.). Hemolymph was placed in 20 ml of 10 mM nitric acid and stored at 5°C. Samples were analyzed for total copper on a Perkin

Elmer Model 703 flameless atomic absorption spectrometer with a HGA-2200 graphite furnace. Values are expressed as μg total copper/ml hemolymph.

Nitrogen excretion rates as well as the atomic ratio of oxygen consumed to nitrogen excreted (O:N ratio) were determined for 6-11 shrimp/treatment. Immediately after measuring whole animal $\dot{V}O_2$ at 20°C, seawater in each respiratory chamber was placed in 4 ml vials, sealed with parafilm and frozen. Subsequently, 1 ml from each thawed sample was removed and analyzed for total ninhydrin positive substances according to Clark (1964). Absorption of samples at 570 nm was determined on a Cary 15 spectrophotometer with glycine as a standard. Nitrogen excretion rates are expressed as μM nitrogen excreted/g dry wt/h while O:N ratios are based on the total μmoles of oxygen consumed to total μmoles of nitrogen excreted.

Water efflux in Palaemonetes pugio was determined by the appearance of tritium label in the external medium following the general procedures of Roesijadi et al. (1976a). Eighteen shrimp/treatment were exposed at 20°C to tritium-labeled seawater (1.39 μ Ci/ml) in four fingerbowls containing 360 ml of 17 °/oo seawater. Tritium-labeled water was obtained from New England Nuclear (specific activity = 1 mCi/g). After 20 h, shrimp were removed, rinsed in 17 °/oo seawater and placed in individual compartments of plastic trays with each compartment containing 20 ml 17 °/oo seawater. Temperature was maintained at 20°C throughout the efflux measurements. The appearance of tritium in the external medium was monitored after

5, 15, 30, 60, 90, 120, 180, 360, 540 and 720 minutes. At each interval, 0.1 ml of seawater was removed and placed in 4 ml plastic scintillation vials each containing 3 ml Quantaflour liquid scintillation cocktail (Mallinckrodt). Samples were counted on a Beckman L-S 150 liquid scintillation counter. Counts were not quench-corrected since quenching was uniform in all samples. The activity at each time interval (C_t) was expressed as a fraction of the final equilibrium activity (C_∞). The natural logarithms of these fractions

$$\left(\ln \frac{C_\infty - C_t}{C_\infty} \right)$$

were regressed against time (h) for each shrimp in individual compartments. From these regressions, rate constants (k) and half-time values ($t_{1/2}$) were calculated according to the formulas,

$$\left(\ln \frac{C_\infty - C_t}{C_\infty} = -kt \right)$$

and

$$t_{1/2} = \frac{0.693}{k}$$ respectively (Comar, 1955).

Non-specific activity of the lysosomal enzyme, acid phosphatase in the hemolymph of Palaemonetes pugio was determined before and after 24 h of hypoxia. Prior to hypoxia, the hemolymph of 9 shrimp from each treatment was withdrawn as described earlier. Ten µl of hemolymph from each shrimp were placed in a 4 ml vial containing 1 ml 17 °/oo seawater. The vials were sealed with parafilm and frozen. Eighteen shrimp from each treatment were exposed to hypoxic conditions (2 mg O_2/ℓ) for 24 h at 20°C. After 24 h of hypoxia, hemolymph from 9-10 shrimp/treatment was obtained and stored as described above.

Preliminary experiments indicated no loss of enzyme activity if samples were analyzed within 1 month of storage. Enzyme activity was determined by the method of Cheng and Butler (1979) except for the use of an acid phosphatase reagent set from Worthington Diagnostics (lot no. 70K376). The substrate, sodium monophosphate, is complexed with thymolphthalein so the reaction may be quantified colorimetrically. After incubating for 30 min at 37°C, the reaction was stopped by adding alkali. Absorption at 590 nm was determined on a Cary 15 spectrophotometer. Non-specific enzyme activity is expressed in International Units (IU)/ℓ or that amount of enzyme required to catalyze 1 µM of substrate/min/ℓ volume.

Dimethylnaphthalene (DMN) Analysis

DMN in tissue and water samples was extracted and analyzed according to the method of Neff and Anderson (1975). Three Artemia sp. food cubes and one 250 ml water sample were analyzed for DMN in three of the Artemia sp. 8 h exposures. Six to 16 grass shrimp/treatment were routinely removed for DMN analysis after the exposure and recovery periods. A more detailed examination of the uptake/depuration profile in grass shrimp was conducted by removing eight shrimp from each treatment after 8, 16, 24 and 32 d exposure and after 8 and 16 d recovery. All tissue samples were frozen on tared aluminum sheets at -20.0°C. After thawing, wet wts were determined just prior to analysis. Grass shrimp were first cut into several pieces with dissecting scissors. Two shrimp were extracted and analyzed together in each sample. Tissues were homogenized by hand in

a consistent manner in the presence of 5 ml spectroscopic grade hexane (Burdick and Jackson Laboratories) with all glass hand homogenizers. The hexane layer was poured into 20 ml glass vials. Approximately 1 g Floricil was added to remove coextracted lipids. Vials were sealed with polyethylene-lined caps and stored at 5°C for no longer than 1 wk. Absorbance at 228 nm was determined on a Cary 15 recording spectrophometer. Concentrations were determined from DMN standards made up in hexane. Tissue interference was corrected by subtracting the absorbance of extracts of uncontaminated tissues. Concentrations are expressed as μg DMN/ℓ water and μg DMN/g wet wt tissue.

Statistical Analysis

Statistical analysis of survival data from the challenge tests was described earlier. For data on the sublethal effects, differences among the four treatment means were compared with a one-way analysis of variance (ANOVA) before and after the recovery period. If indicated, mean comparison was performed according to the Student-Newman-Keul's test (Steele and Torrie, 1960). All significant differences were examined at the 95% confidence level or $\alpha = 0.05$.

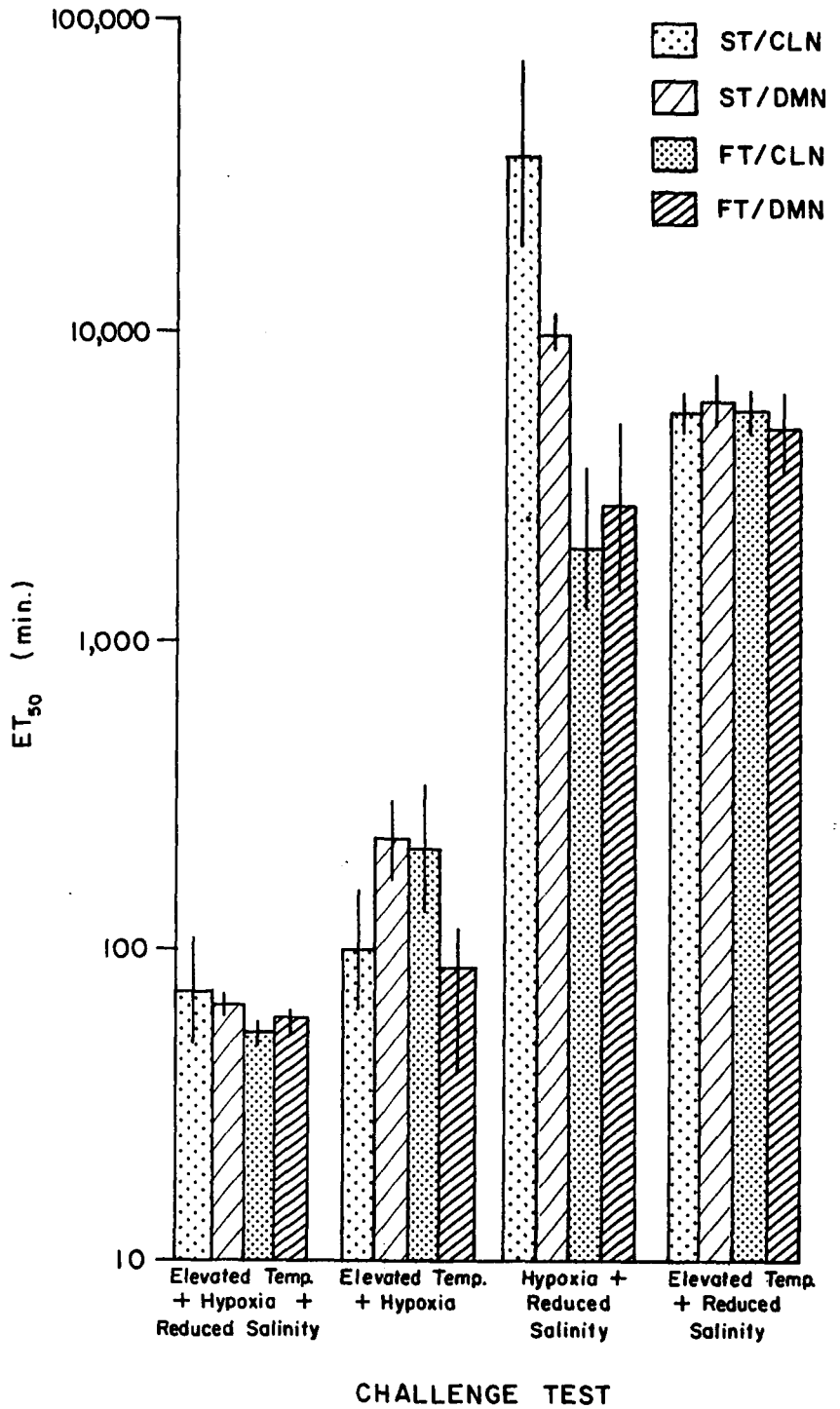
RESULTS

Survival to Environmental Challenge

Survival times for Palaemonetes pugio in the challenge tests of elevated temperature + hypoxia both with and without reduced salinity were significantly less than in the challenges of hypoxia + reduced salinity or elevated temperature + reduced salinity (Figure 1). In the former two challenges the mean ET_{50}'s ranged from about 60-200 minutes while in the latter two the mean ET_{50}'s ranged from about 4,600-13,600 minutes. The four treatments appear to affect the survival of shrimp differently in the two challenge tests of hypoxia + elevated temperature and hypoxia + reduced salinity. The response to hypoxia + elevated temperature appears to be a complex one with no readily discernible patterns. This may be due to the rapidity with which death occurred. Mean ET_{50}'s ranged from about 90-220 minutes in the challenge test.

A clear pattern does seem evident in the survival of shrimp to the challenge of hypoxia + reduced salinity, in which survival times ranged from about 2,000-13,600 minutes. The response to this challenge was examined with an analysis designed specifically for time-percent effect experiments (Litchfield, 1949). The results of that analysis indicated that the least resistant groups of shrimp among the four treatments are those which were exposed to fluctuating temperatures (FT), independent of diet. Mean ET_{50}'s for shrimp in the

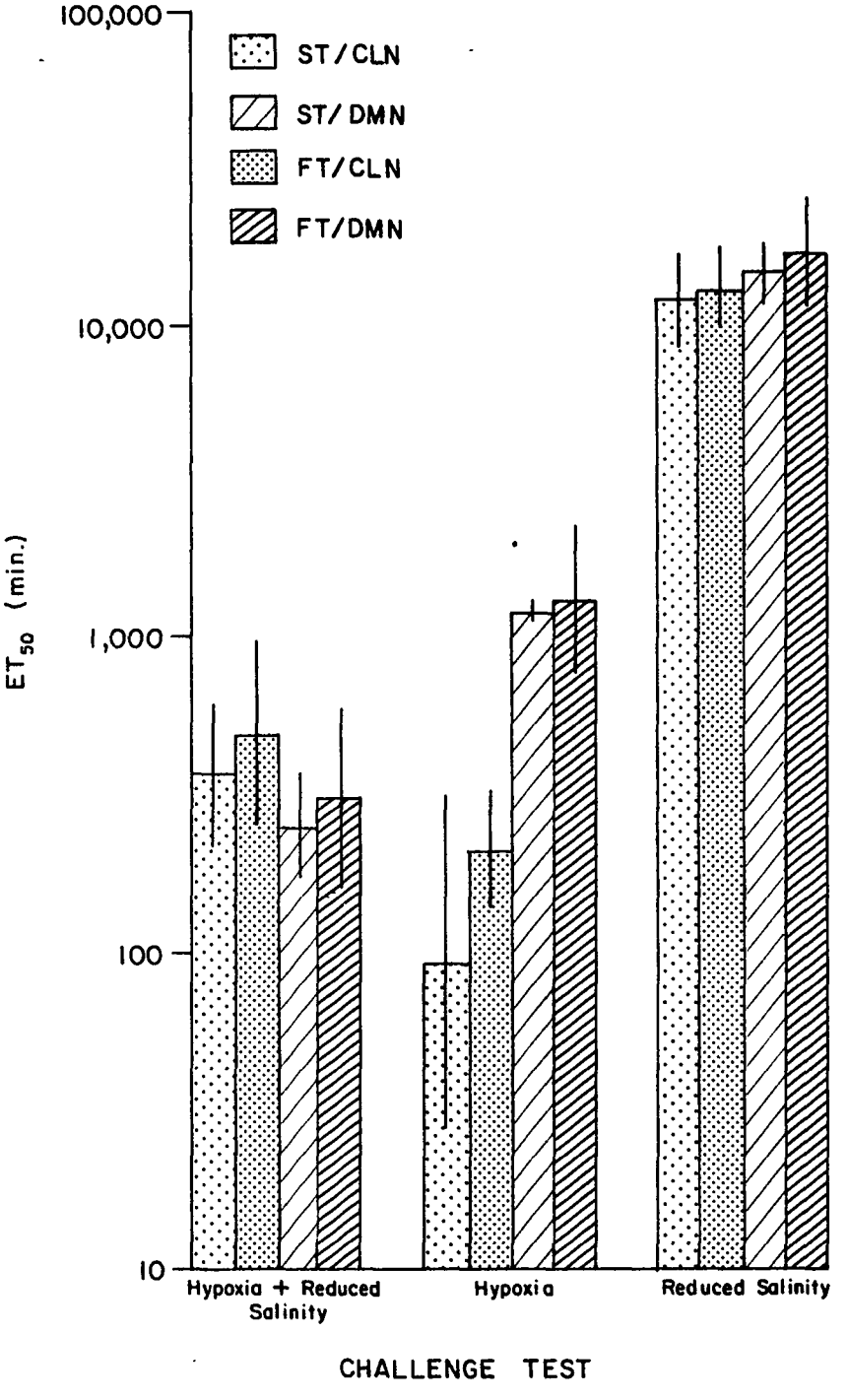
Figure 1. Effects of the four experimental treatments on the estimated time to 50% mortality (ET_{50}) of Palaemonetes pugio in challenge tests after the 32 d exposure period. Vertical bars represent 95% confidence intervals. 18 shrimp/challenge test.


100,000
10,000
1,000
100
10
ET50 (min.)
ST/CLN
ST/DMN
FT/CLN
FT/DMN
Elevated Temp. + Hypoxia + Reduced Salinity
Elevated Temp. + Hypoxia
Hypoxia + Reduced Salinity
Elevated Temp. + Reduced Salinity
CHALLENGE TEST

FT/CLN and FT/DMN regimes ranged from about 2,000-2,600 minutes. Shrimp transferred from the stable temperature regimes and fed DMN-contaminated food did not survive hypoxia as well as those fed clean food. The mean ET_{50} of shrimp from the ST/DMN regimes was 9,500 minutes while the mean ET_{50} of shrimp from the ST/CLN regime was 13,600 minutes.

Because FT and contaminated food significantly affected the shrimps' survival to hypoxia + reduced salinity, it was decided to examine this challenge in greater detail after the 16 day recovery period. The effects of the four treatments after the recovery period do not appear to affect the survival of Palaemonetes pugio to the challenge tests of hypoxia + reduced salinity or reduced salinity but do affect survival in the hypoxia challenge test (Figure 2). Comparison of ET_{50}'s from this latter test (Litchfield, 1949) reveals two significant results. First, the effects of FT do not affect survival to hypoxia. Second, shrimp which ingested nothing but uncontaminated food do not survive hypoxia as well as shrimp which had previously ingested DMN-contaminated food. Mean ET_{50}'s ranged from about 90-200 minutes in the former group of shrimp and 1,200-1,300 minutes in the latter. Results of the ANOVA indicated that the challenge of reduced salinity was less stressful (mean ET_{50}'s = about 1,400 minutes) than the challenges of hypoxia + reduced salinity, (mean ET_{50}'s ranged from 250-410 minutes) or hypoxia alone (mean ET_{50}'s ranged from 90-1,300 minutes). Because shrimp were not fed during the challenge tests, it is uncertain to what extent starvation

Figure 2. Effects of the four experimental treatments on the estimated time to 50% mortality (ET_{50}) of Palaemonetes pugio in challenge tests after the 16 d recovery period. Vertical bars represent 95% confidence intervals. 15 shrimp/challenge test.


100,000
10,000
1,000
100
10
ST/CLN
ST/DMN
FT/CLN
FT/DMN
ET50 (min.)
Hypoxia + Reduced Salinity
Hypoxia
Reduced Salinity
CHALLENGE TEST

affected survival, especially in groups of shrimp with more protracted survival.

Sublethal Effects on Oxygen Consumption Rates ($\dot{V}O_2$)

The challenge tests indicated that the effects of FT and DMN-contaminated food affected the ability of Palaemonetes pugio to survive hypoxic conditions differently. For this reason, $\dot{V}O_2$ was determined under a variety of conditions. After the 32 day exposure period, there were no significant differences due to the four treatments in either whole animal or dissected tissue $\dot{V}O_2$ at 20°C (Figure 3). Mean rates ranged from 2.1-2.3 µl O_2/mg dry wt/h for whole animal oxygen consumption and from 0.6-0.7 µl O_2/mg dry wt/h for dissected tissue.

FT and DMN-contaminated food had no significant effect on whole animal or dissected tissue $\dot{V}O_2$ at 20°C after the recovery period (Figure 3). Absolute rates after the recovery period were very similar to those observed after the exposure period. Mean $\dot{V}O_2$ after the recovery period ranged from 2.0-2.2 µl O_2/mg dry wt/h for whole animals and 0.6-0.7 µl O_2/mg dry wt/h for dissected tissues.

There was a significant difference in mean hemolymph copper (Cu) concentrations among shrimp in the four treatments after the exposure period (Figure 4). Subsequent mean separation, via Student-Newman-Kuel's, showed that the mean Cu concentration in shrimp from the FT/DMN regime (57.8 µg Cu/ml hemolymph) was not significantly lower than that of shrimp in the ST/DMN regime (69.8 µg Cu/ml hemolymph) but

Figure 3. Effects of the four experimental treatments on whole animal and dissected tissue oxygen consumption rates ($\dot{V}o_2$) at 20°C after the 32 d exposure and 16 d recovery periods. Vertical bars represent $\pm$ 1 standard error of the mean. Numbers in parentheses = number of determinations/mean.

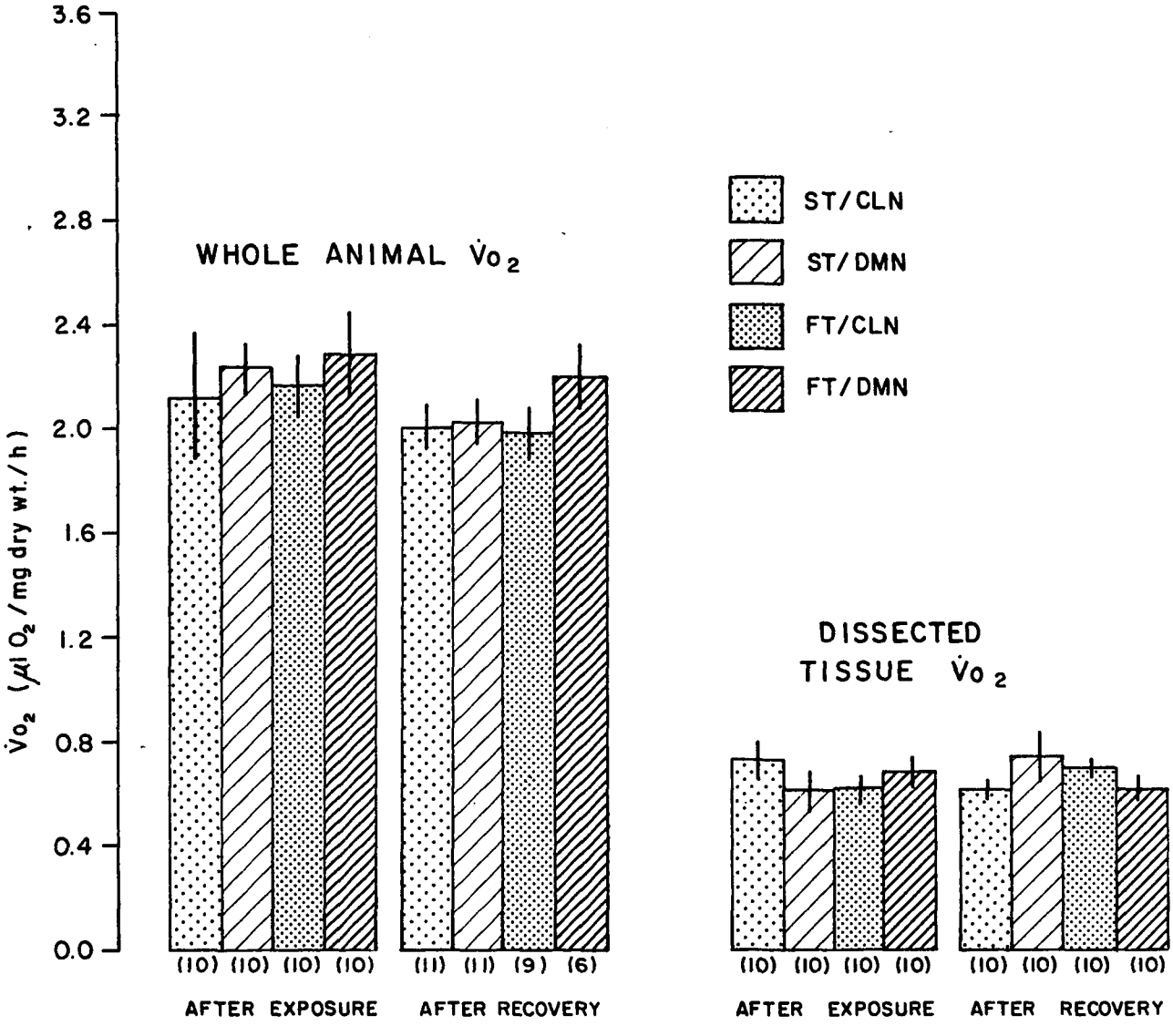
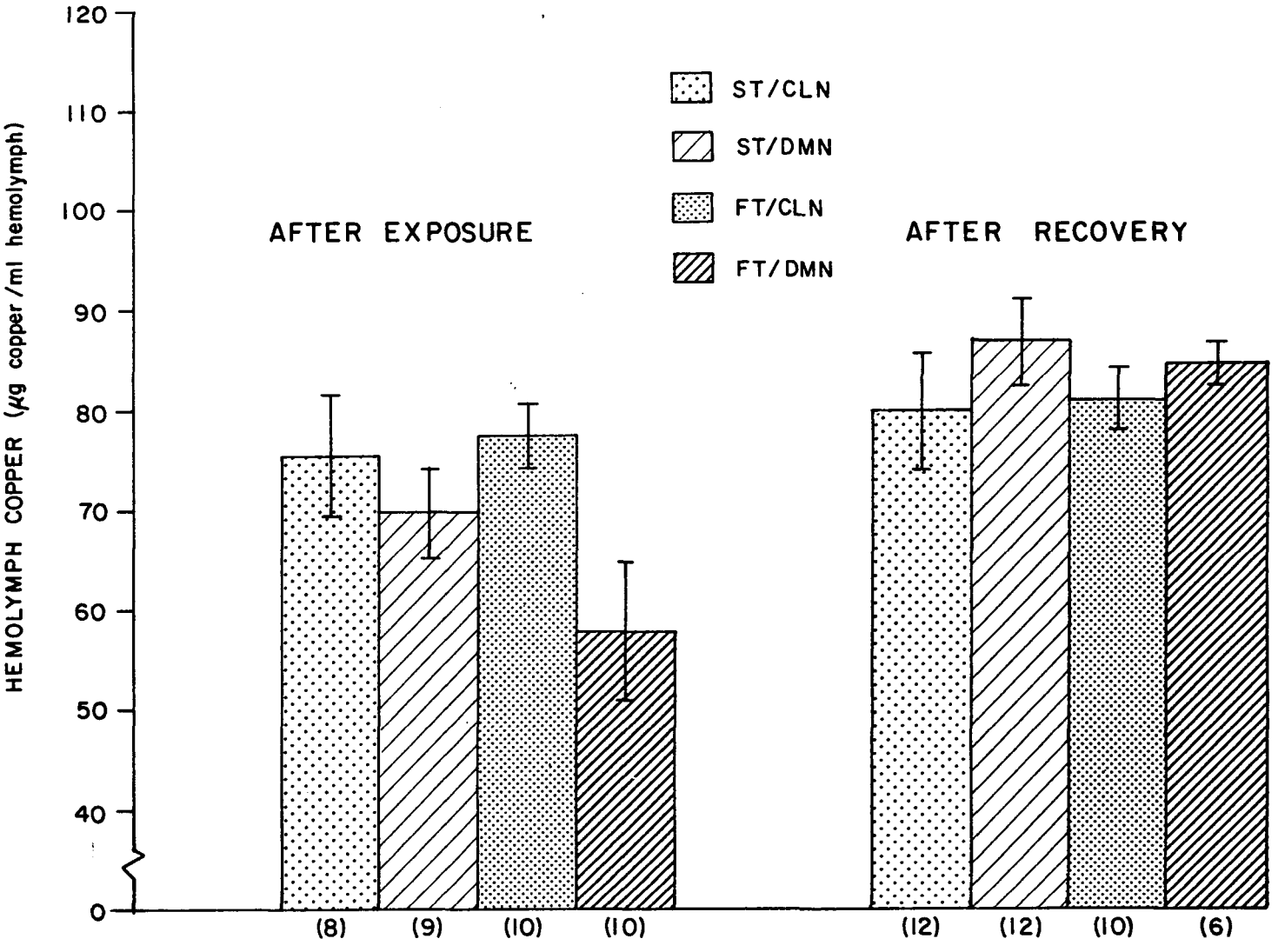

3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
$\dot{V}o_2$ (μl O_2 / mg dry wt. / h)
WHOLE ANIMAL $\dot{V}o_2$
ST/CLN
ST/DMN
FT/CLN
FT/DMN
(10) (10) (10) (10) (11) (11) (9) (6)
AFTER EXPOSURE
AFTER RECOVERY
DISSECTED TISSUE $\dot{V}o_2$
(10) (10) (10) (10) (10) (10) (10) (10)
AFTER EXPOSURE
AFTER RECOVERY

Figure 4. Effects of the four experimental treatments on hemolymph copper concentrations after the 32 d of exposure and 16 d recovery periods. Vertical bars represent $\pm$ 1 standard error of the mean. Numbers in parentheses = number of determinations/mean.


HEMOLYMPH COPPER (µg copper /ml hemolymph)
120
110
100
90
80
70
60
50
40
0
ST/CLN
ST/DMN
FT/CLN
FT/DMN
AFTER EXPOSURE
AFTER RECOVERY
(8)
(9)
(10)
(10)
(12)
(12)
(10)
(6)

was significantly lower than mean concentrations in shrimp from the ST/CLN (75.6 µg Cu/ml hemolymph) and FT/CLN (77.6 µg Cu/ml hemolymph) regimes. Mean Cu concentrations in shrimp from these latter two regimes were not significantly different from that of shrimp from the ST/DMN regime. After the recovery period, there were no significant differences in mean Cu concentrations among shrimp from the four treatments (Figure 4). Mean values after the recovery period ranged from 79.5-86.8 µg Cu/ml hemolymph.

The relationship between $\dot{V}o_2$ and declining oxygen concentrations at 20°C was found to be linear with correlation coefficients ranging from 0.911-0.970 (Table 2). The mean linear estimates from Table 2 were used to reconstruct regressions for each group of shrimp (Figure 5). Shrimp in both the ST/CLN and FT/CLN regimes appear to respond to declining oxygen concentrations in a similar fashion before and after recovery. However, for shrimp ingesting DMN-contaminated food, the response to declining oxygen appears to be different after the recovery period. Shrimp ingesting DMN-contaminated food had significantly greater slopes (means ranged from 2.50-2.55) than shrimp ingesting uncontaminatd food (means ranged from 1.43-1.52) regardless of the thermal regimes. After the recovery period, there were no significant differences in slopes among the four experimental groups in which the means ranged from 1.03-1.52.

After the 32 day exposure period there were no significant differences in $\dot{V}o_2$ at either 15° or 25°C due to the four experimental treatments (Figure 6). Previously reported $\dot{V}o_2$ at 20°C (Figure 3)

Table 2. Mean linear regression estimates of $\dot{V}O_2$ at 20°C of Palaemonetes pugio in declining oxygen concentrations after the exposure and recovery periods. Mean ± 1 SE n = number of replicate regressions.

	n	Correlation Coefficient	Y - Intercept	Slope
After Exposure				
ST/CLN	3	0.911 ± 0.016	0.103 ± 0.021	1.43 ± 0.24
ST/DMN	3	0.924 ± 0.035	0.065 ± 0.020	2.55 ± 0.48
FT/CLN	3	0.932 ± 0.004	0.113 ± 0.014	1.52 ± 0.06
FT/DMN	3	0.950 ± 0.010	0.052 ± 0.090	2.50 ± 0.16
After Recovery				
ST/CLN	6	0.930 ± 0.029	0.106 ± 0.028	1.52 ± 0.22
ST/DMN	6	0.938 ± 0.013	0.067 ± 0.007	1.03 ± 0.16
FT/CLN	6	0.970 ± 0.008	0.053 ± 0.014	1.51 ± 0.27
FT/DMN	6	0.963 ± 0.005	0.048 ± 0.009	1.32 ± 0.17

Figure 5. Effects of the four experimental treatments on oxygen comsumption rates ($\dot{V}o_2$) in declining oxygen concentrations at 20°C after the 32 d exposure and 16 d recovery periods. Each line represents the mean of 3 regressions after exposure and 6 regressions after recovery.

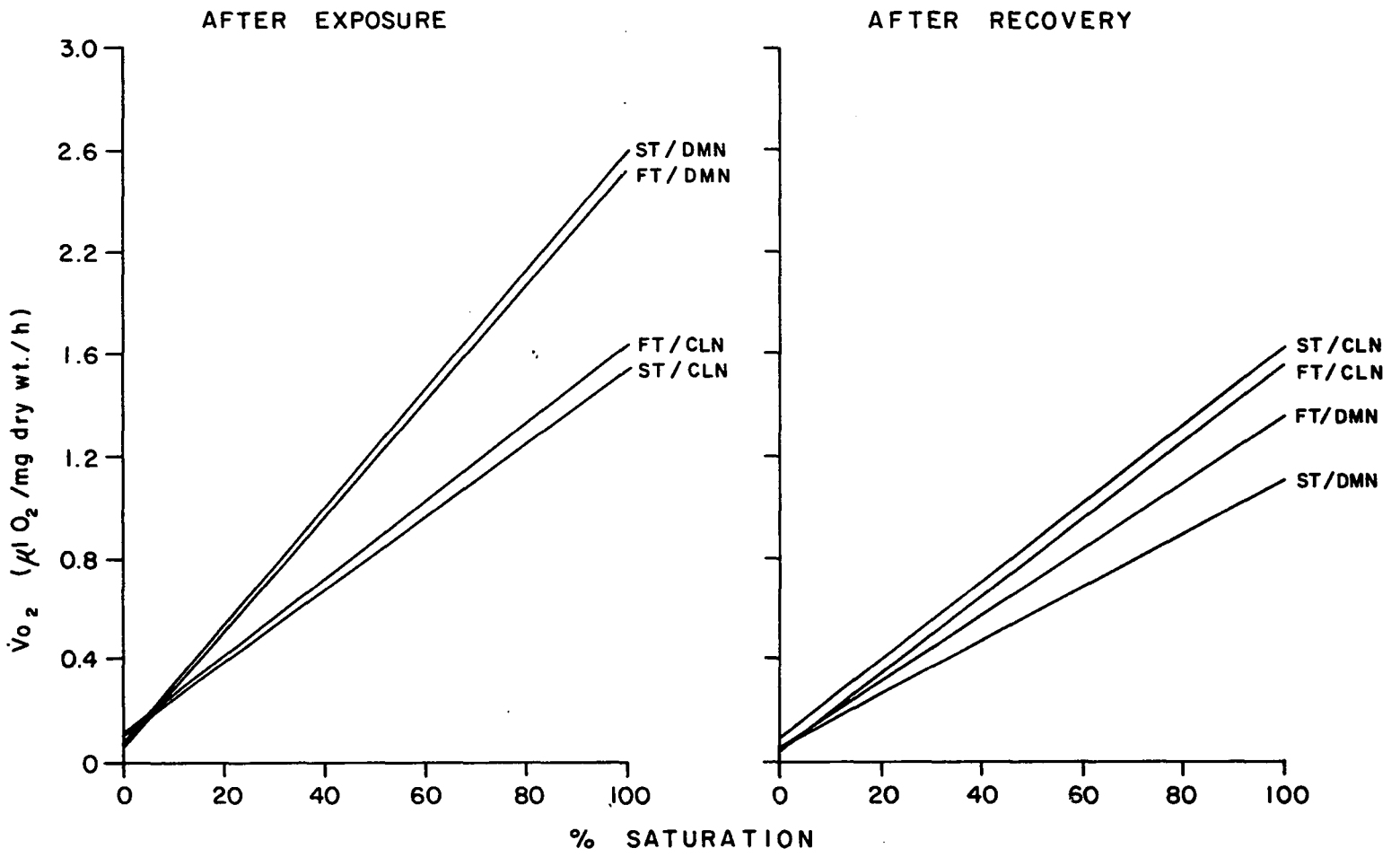
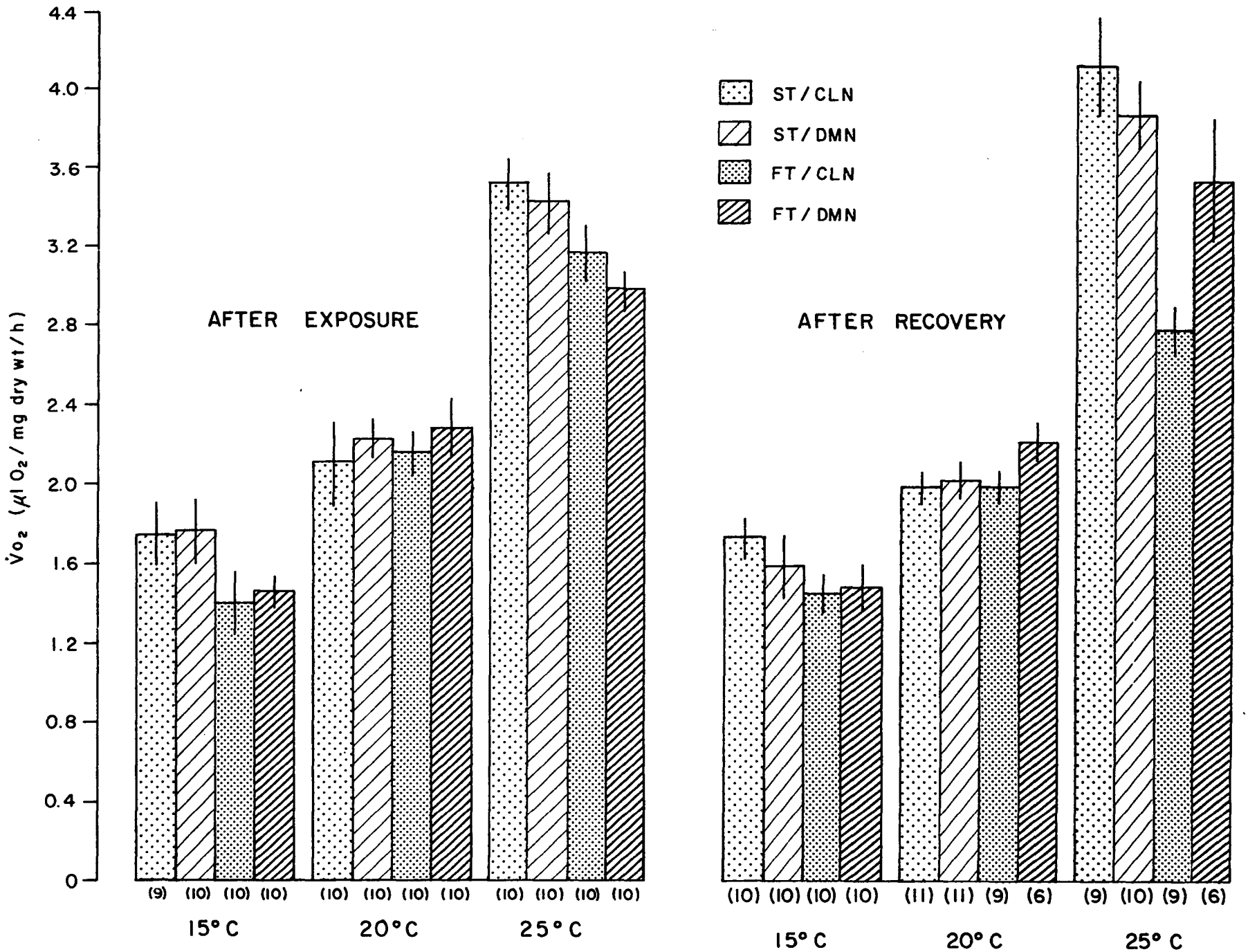

AFTER EXPOSURE
AFTER RECOVERY
3.0
2.6
2.2
1.6
1.2
0.8
0.4
0
$\dot{V}o_2$ (μl O_2 /mg dry wt./h)
ST / DMN
FT / DMN
FT / CLN
ST / CLN
ST / CLN
FT / CLN
FT/DMN
ST/DMN
0
20
40
60
80
100
% SATURATION

Figure 6. Effects of the four experimental treatments oxygen consumption rates ($\dot{V}O_2$) at 15°C, 20°C and 25,C after the 32 d exposure and 16 d recovery periods. Vertical bars represent $\pm$ 1 standard error of the mean. Numbers in parentheses = number of determinations/mean.


4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
$\dot{V}O_2$ (μl O_2 / mg dry wt / h)
AFTER EXPOSURE
AFTER RECOVERY
ST / CLN
ST / DMN
FT / CLN
FT / DMN
(9) (10) (10) (10)
(10) (10) (10) (10)
(10) (10) (10) (10)
15° C
20° C
25° C
(10) (10) (10) (10)
(11) (11) (9) (6)
(9) (10) (9) (6)
15° C
20° C
25° C

have been included for comparative purposes. Mean $\dot{V}o_2$ ranged from about 1.4-1.7 µl O_2/mg dry wt/hr in 15°C and from 3.0-3.5 µl O_2/mg dry wt/hr in 25°C. There does appear to be a consistent trend of slightly depressed respiratory rates in shrimp from the FT regimes experiencing an acute change in temperature. However, these values were not significantly lower than mean $\dot{V}o_2$ of shrimp from the stable temperature regimes.

$\dot{V}o_2$ in response to a change in temperature after the recovery period were very similar to those observed after exposure with mean values ranging from 1.4-1.7 µl O_2/mg dry wt/h in 15°C and 2.7-4.1 µl O_2/mg dry wt/h in 25°C. However, the residual effects of FT act to significantly depress Vo_2 of shrimp acutely exposed to 25°C. The mean $\dot{V}o_2$ of shrimp from the FT/CLN regime was about 2.8 µl O_2/mg dry wt/h while in the ST/CLN regime it was 4.1 µl O_2/mg dry wt/h. This depression of $\dot{V}o_2$ at 25°C due to the residual effects of FT is offset by the stimulatory effect of DMN-contaminated food. The mean $\dot{V}o_2$ of shrimp from the FT/DMN regime was about 3.5 µl O_2/mg dry wt/h while in the ST/DMN regime it was about 3.8 µl O_2/mg dry wt/h. The residual effects of FT do not affect $\dot{V}o_2$ of shrimp acutely exposed to 15°C. Mean $\dot{V}o_2$ of shrimp in 15°C ranged from about 1.4-1.7 µl O_2/mg dry wt/h.

Physiological Indices of Stress

Shrimp from FT/DMN had greater mean O:N ratios after both the exposure (64.3) and recovery (12.3) periods than mean O:N ratios for shrimp in the other three treatments which were not significantly

different from one another and ranged from 25-35 and from 2.7-2.8 after the exposure and recovery periods, respective (Figure 7). Within each treatment, O:N ratios were lower after the recovery period (means ranged from 22.3-64.3) than after the exposure period in which means ranged from 2.7-12.3. These low O:N ratios after the recovery period appear to result from elevated nitrogen excretion rates in shrimp from all four treatments (Figure 9). Shrimp ingesting DMN-contaminated food had significantly lower mean nitrogen excretion rates after the exposure period (about 2 μmoles nitrogen/g dry wt/h) relative to shrimp ingesting uncontaminated food (about 4.6 μmoles nitrogen/g dry wt/h).

Shrimp from the FT/CLN regime have significantly lower water efflux rates both before and after recovery as evidenced by significantly higher mean half-times (0.43 to 0.67 h) and lower mean rate constants (-1.33 to -1.72 h^{-1}) compared to shrimp in the other three treatments in which mean half-times ranged from 0.34 to 0.45 h and mean rate constants from -1.61 to 2.28 h^{-1} (Table 3). Water efflux rates increased uniformly in all treatments after the recovery period. After the exposure period mean half-times ranged from 0.43 to 0.67 h and rate constants from -1.33 to -1.84 h^{-1} while after the recovery period mean half-times and rate constants ranged from 0.34 to 0.43 h and -1.72 to -2.28, respectively.

After the exposure period, hypoxia induced a significantly greater level of acid phosphatase activity in the hemolymph of shrimp from the FT regimes (means ranging from 1.77-2.08 IU/l), regardless of

Figure 7. Effects of the four experimental treatments on the ratio of oxygen consumed to nitrogen excreted (O:N ratio) at 20°C after the 32 d exposure and 16 d recovery periods. Vertical bars represent ± 1 standard error of the mean. Numbers in parentheses = number of determinations/mean.

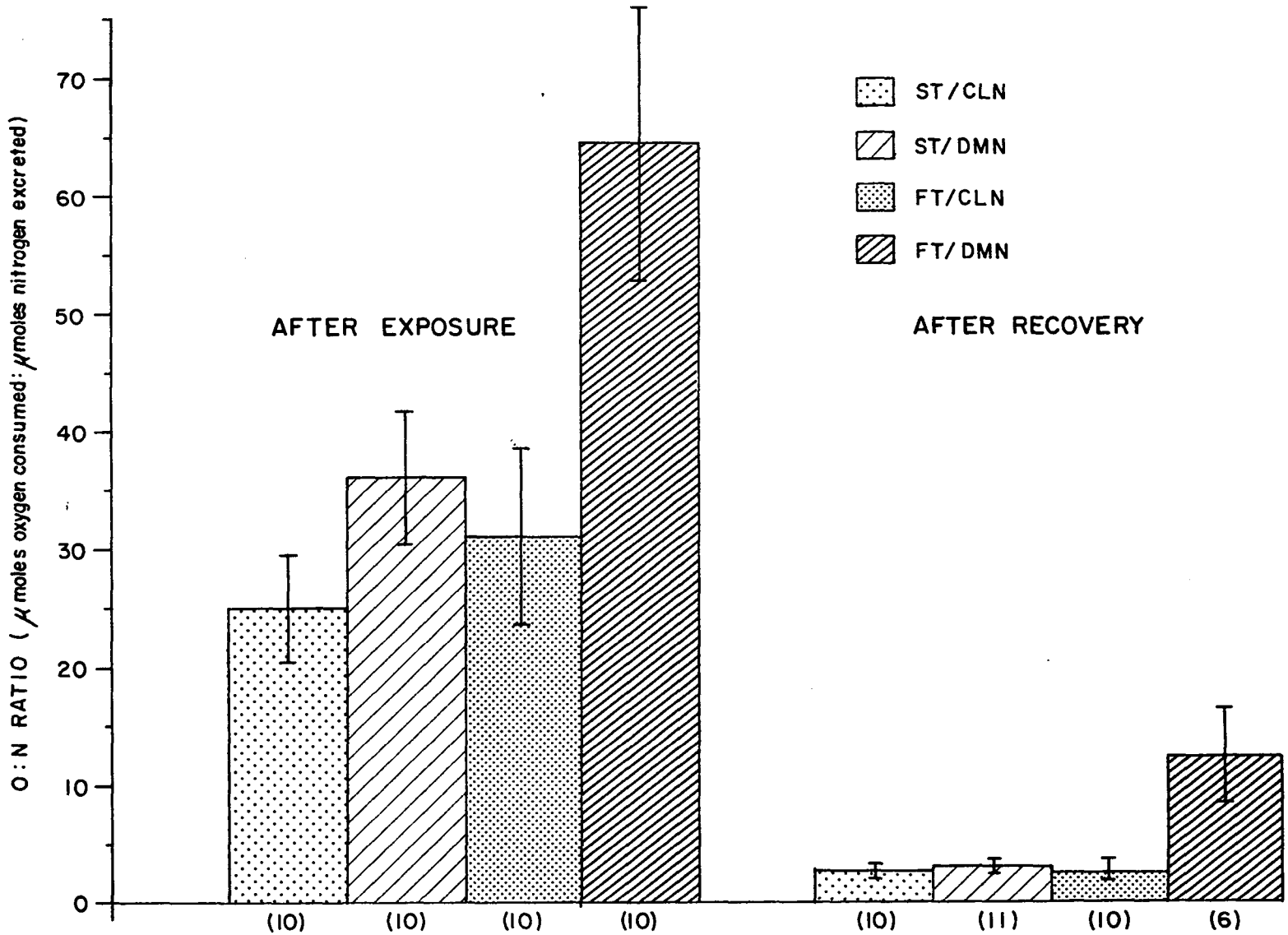

O:N RATIO (μmoles oxygen consumed: μmoles nitrogen excreted)
70
60
50
40
30
20
10
0
ST/CLN
ST/DMN
FT/CLN
FT/DMN
AFTER EXPOSURE
AFTER RECOVERY
(10)
(10)
(10)
(10)
(10)
(11)
(10)
(6)

Figure 8. Effects of the four experimental treatments on nitrogen excretion rates at 20°C after the 32 d exposure and 16 d recovery periods. Vertical bars represent $\pm$ 1 standard error of the mean. Numbers in parentheses = number of determinations/mean.

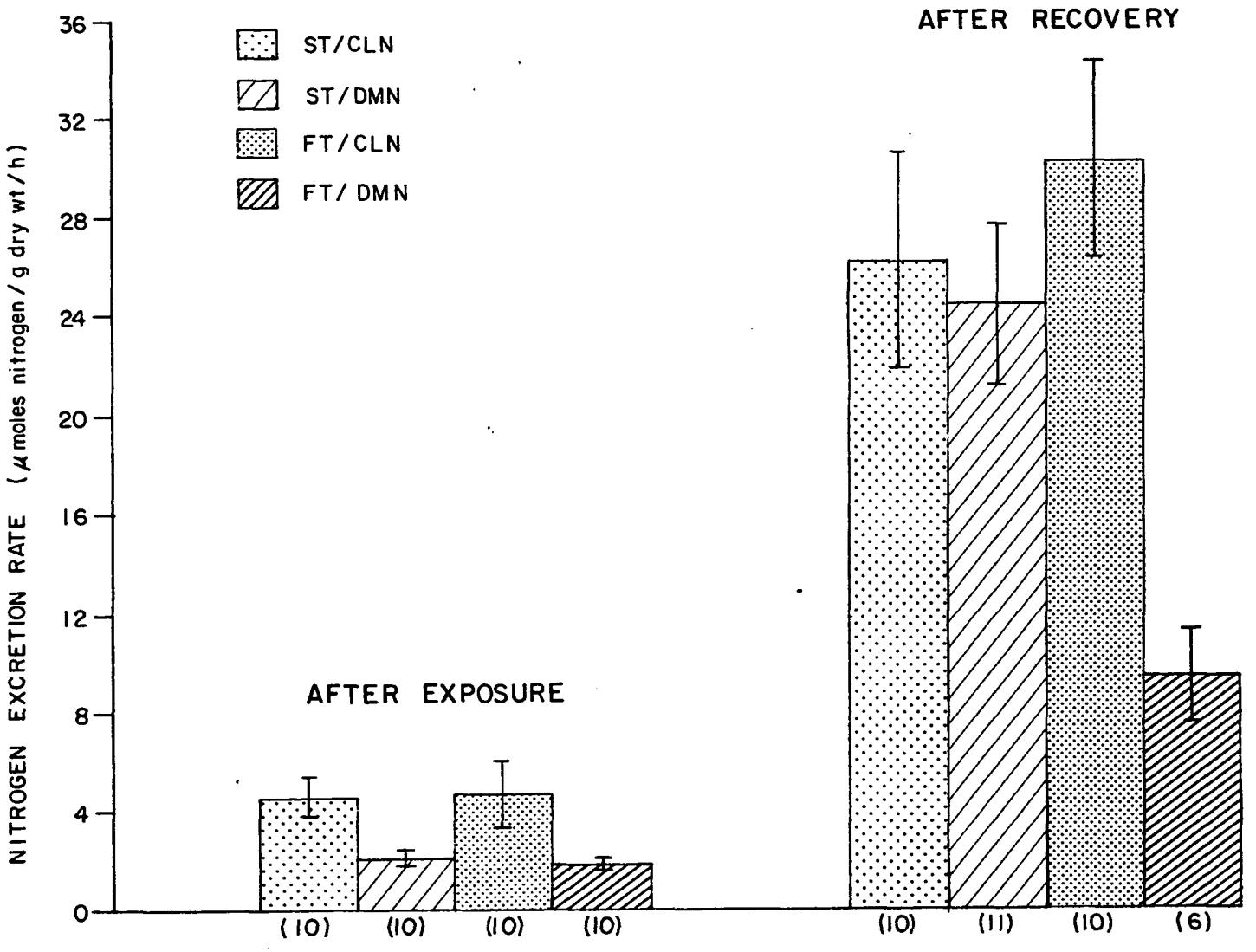

AFTER RECOVERY
ST/CLN
ST/DMN
FT/CLN
FT/ DMN
NITROGEN EXCRETION RATE (μ moles nitrogen / g dry wt / h)
36
32
28
24
20
16
12
8
4
0
AFTER EXPOSURE
(10)
(10)
(10)
(10)
(10)
(11)
(10)
(6)

Table 3. Water efflux parameters for Palaemonetes pugio at 20°C after the exposure and recovery periods. $t_{1/2}$ = half-times. k = rate constant. Mean ± 1 SE n = number of shrimp.

	$t_{1/2}$ (h)		k (h^{-1})	
	After Exposure	After Recovery	After Exposure	After Recovery
ST/CLN	0.44 ± 0.02 n=14	0.38 ± 0.02 n=18	1.61 ± 0.06 n=14	2.05 ± 0.20 n=18
ST/DMN	0.45 ± 0.02 n=16	0.37 ± 0.02 n=18	1.61 ± 0.08 n=16	1.93 ± 0.09 n=18
FT/CLN	0.67 ± 0.09 n=17	0.43 ± 0.02 n=17	1.33 ± 0.14 n=17	1.72 ± 0.15 n=17
FT/DMN	0.43 ± 0.04 n=18	0.34 ± 0.03 n=18	1.84 ± 0.15 n=18	2.28 ± 0.17 n=18

diet, relative to shrimp from the stable temperature regimes in which means ranged from 0.93-1.02 IU/l (Figure 9). After the recovery period a different pattern was observed. Prior to hypoxia, shrimp from the two FT regimes had higher levels of enzyme activity (means ranging from 1.04-1.24 IU/l) compared to shrimp from the stable temperature regimes in which means ranged from 0.57-0.72 IU/l. Following 24 h of hypoxia, shrimp from the stable temperature and FT regimes, which had ingested nothing but uncontaminated food, had elevated levels of activity in their hemolymph (means ranging from 1.66-1.81 IU/l) relative to shrimp which had ingested DMN-contaminated food (means ranging from 1.09-1.16 IU/l).

Dimethylnaphthalene (DMN) Analysis

There were no significant differences in the concentration of DMN in shrimp among any of the exposure/recovery periods. Consequently, overall means were calculated within each treatment after the exposure and recovery periods (Table 4). After ingesting contaminated food for 32 days, Palaemonetes pugio accumulated approximarely an order of magnitude greater concentration of DMN than in the Artemia sp. food cubes (0.24 µg DMN/g wet wt). The overall means of DMN in shrimp from the FT and stable temperature regimes were 5.26 and 7.20 µg DMN/g wet wt, respectively. Results of the two-way ANOVA indicated that there was a significant decrease in DMN concentrations for shrimp in both stable temperatures and FT after the recovery period to levels of 1.27 and 2.60 µg DMN/g wet wt, respectively. There were no significant

Figure 9. Effects of the four experimental treatments on non-specific acid phosphatase activity in the hemolymph of shrimp after the 32 d exposure and 16 d recovery periods. Activities were determined before and after a 24 h exposure to hypoxic conditions (2 mg O_2/ℓ) at 20°C. Vertical bars represent $\pm$ 1 standard error of the mean. Numbers in parentheses = number of determinations/mean.

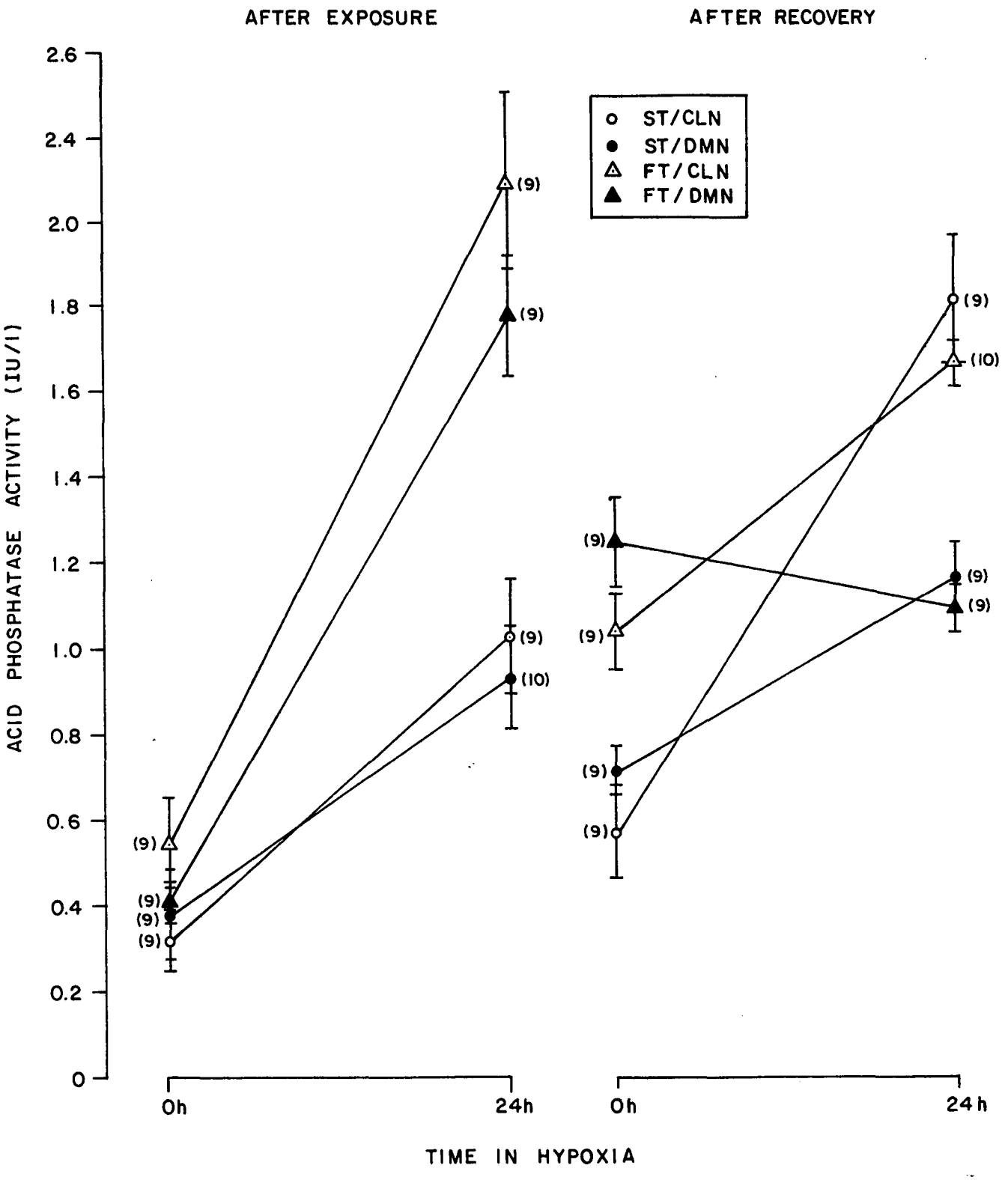

AFTER EXPOSURE
AFTER RECOVERY
ST/CLN
ST/DMN
FT/CLN
FT/DMN
ACID PHOSPHATASE ACTIVITY (IU/l)
2.6
2.4
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
(9)
(9)
(9)
(9)
(9)
(9)
(10)
(9)
(9)
(9)
(9)
(9)
(10)
(9)
(9)
0h
24h
0h
24h
TIME IN HYPOXIA

Table 4. Concentrations of dimethylnaphthalene (DMN) (µg DMN/g wet wt.) in *Artemia* sp. and *Palaemonetes pugio* from all exposure and recovery periods. $\bar{x} \pm 1$ SE n = number of samples analyzed.

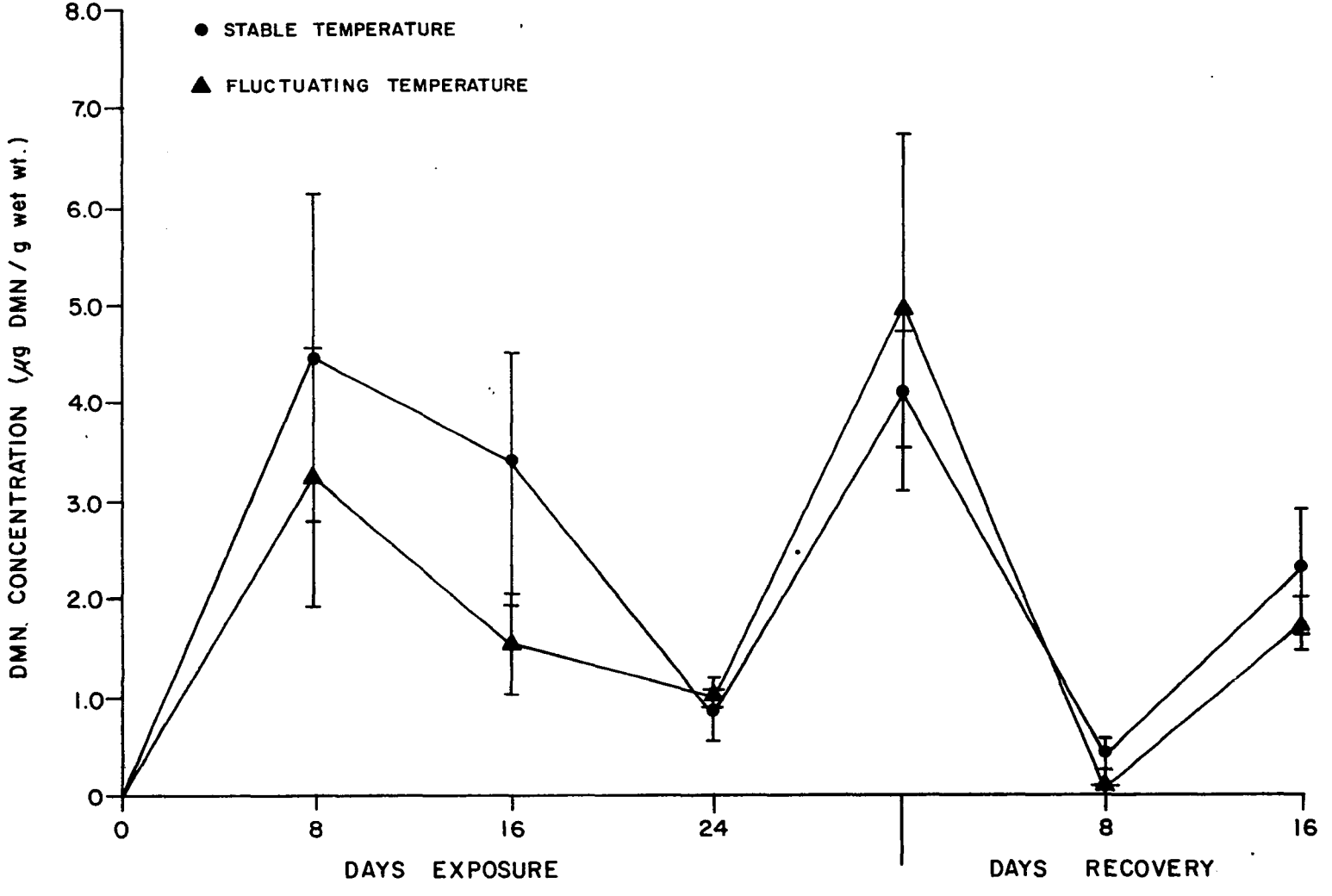
		Palaemonetes pugio[b]	*Artemia* sp.[c]
Exposure			
ST	$\bar{x} \pm$ SE	5.26[a] ± 1.37 n=20	0.24 ± 0.06 n=9
FT	$\bar{x} \pm$ SE	7.20[a] ± 1.00 n=20	0.24 ± 0.06 n=9
Recovery			
ST	$\bar{x} \pm$ SE	1.27[a] ± 0.35 n=27	0.00[d]
FT	$\bar{x} \pm$ SE	2.60[a] ± 0.48 n=29	0.00[d]

[a] overall means
[b] each sample analyzed contained two shrimp
[c] each sample analyzed contained one food cube
[d] uncontaminated food source

differences in DMN concentrations in shrimp from the two thermal treatments either after exposure or recovery.

Similar patterns of uptake and depuration of DMN during the course of the 32 day exposure and the 16 day recovery periods are observed for shrimp from the stable temperature and FT regimes (Figure 10). Mean tissue concentrations of DMN in Palaemonetes pugio ranged from 3.27-4.45 μg DMN/g wet wt after 8 days. There was an equally rapid decrease in tissue contamination after ingesting uncontaminated food for 8 days with mean values ranging from 0.17-0.36 μg DMN/g wet wt. There is, however, considerable variation in the uptake and depuration period which is inconsistent with the constant daily feeding regime. Preliminary experiments indicated that variation in DMN concentrations associated with the extraction/analysis procedure were smaller than the variation in DMN concentrations among shrimp.

Figure 10. Effects of stable (20°C) and fluctuating (18°-22°C) temperatures on dimethylnaphthalene (DMN) concentrations during the 32 d exposure and 16 d recovery periods. Vertical bars represent $\pm$ 1 standard error of the mean. 4 determinations/mean.


STABLE TEMPERATURE
FLUCTUATING TEMPERATURE
DMN CONCENTRATION (µg DMN / g wet wt.)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0
8
16
24
8
16
DAYS EXPOSURE
DAYS RECOVERY

DISCUSSION

Survival to Environmental Challenge

According to Bayne's (1975) definition of stress in marine invertebrates (see Introduction) daily fluctuating temperatures (FT) were quantitatively more stressful to Palaemonetes pugio than the ingestion of dimethylnaphthalene (DMN)-contaminated food. This was evidenced by a reduction in survival of Palaemonetes pugio subjected to an environmental challenge. It was somewhat surprising that such a small range of daily temperature fluctuation (4°C) near the middle of its tolerance range would have such a significant impact on Palaemonetes pugio. This range of temperatures is representative of daily temperature variations occurring in the environment from which these shrimp were collected (Moore, 1974; Mangum, 1976). Other studies on the effects of FT on marine organisms have generally utilized a large variation in temperatures ranging from 5°-10°C (Regnault and Costlow, 1970; Dame and Vernberg, 1978; Lucas and Costlow, 1979; Sastry, 1979; Huppert and Laudien, 1980; Thorp and Wineriter, 1981). Thorp and Hoss (1975) exposed Palaemonetes pugio and P. vulgaris to FT (7°-13°C) at 20 °/oo and 35 °/oo for 21 d and found no effect on survival due to FT for either of the two sympatric species. They did report reduced survival due to FT when shrimp were maintained in 5 °/oo seawater or when shrimp were subjected to an acute change in temperature (2°C). The effects of FT on survival of shrimp to hypoxia were not evaluated.

Although less stressful, the ingestion of DMN-contaminated food did reduce survival under hypoxic conditions for shrimp held at stable temperatures. Reduced survival under hypoxia has been observed for fish exposed to oiled water (Tagatz, 1961). Rossi and Anderson (1977) reported similar results for the marine worm, Neanthes arenaceodentata exposed to water-soluble fractions of oil. However, they found no corresponding inhibition of increasing hemoglobin concentrations, a normal response to hypoxia by these worms. The effects of ingesting petroleum-contaminated food on the subsequent survival of marine organisms to hypoxia have not been reported before.

Palaemonetes pugio inhabits organically rich estuarine environments which periodically experience low oxygen conditions (Moore, 1974; Taft et al., 1980; Webb and D'Elia, 1980). Consequently, any reduction in the ability of Palaemonetes pugio to survive hypoxic conditions may have direct ecological consequences. Welsh (1975) suggested that it is the ability of Palaemonetes pugio to survive hypoxic conditions which allows it to play such a vital role in the cycling of nutrients and energy in the ecosystem as well as to escape many of its more hypoxia-sensitive predators.

The effects of FT and DMN-contaminated food on the survival to the challenge tests after the recovery period, in which uncontaminated food was provided in a stable temperature environment, forces one to reevaluate the significance of these two perturbations. The results indicate that if these stresses are removed, Palaemonetes pugio can recover. After the recovery period, the residual effects of FT do not

alter survival to hypoxia compared to shrimp from the stable temperature regimes. However, shrimp which had ingested DMN-contaminated food, somewhat surprisingly, exhibit enhanced survival relative to those shrimp which had ingested nothing but uncontaminated food. How can these results be interpreted in terms of actual conditions in the estuarine environment? If the source of contaminated food is eliminated, or if a non-contaminated food source becomes available to the shrimp, results from this study indicate an improved, if not enhanced, survival for grass shrimp exposed to hypoxic conditions.

The recovery of shrimp from the detrimental effects of FT, after a period in stable temperatures, is somewhat difficult to interpret since FT, unlike inputs of petroleum, are inherent characteristics of estuaries and cannot be eliminated. Thermal preferences have been observed for crustaceans (Hall et al., 1978b; Reynolds and Casterlin, 1979) as well as for poikilotherms in general (Hutchinson and Maness, 1979). If the effects of FT were unfavorable to Palaemonetes pugio, one may assume that they would be able to seek an environment in which the thermal regime was less variable. Results of the present study indicate that survival to hypoxia would be improved if shrimp selected a more stable temperature environment.

Sublethal Effects on Oxygen Consumption ($\dot{V}o_2$)

After the exposure period, DMN-contaminated food diminished survival of Palaemonetes pugio under hypoxic conditions. The only respiratory measurement which correlated with this diminished survival

was the relationship between $\dot{V}O_2$ and declining oxygen concentrations. For all shrimp, this relationship was linear, indicating grass shrimp are oxygen conformers. Welsh (1975) found a similar linear response in the $\dot{V}O_2$ of Palaemonetes pugio exposed to declining oxygen in closed respiratory chambers. deFur and Mangum (1979) have shown that the normal cardiac response to declining oxygen tensions, for a variety of marine invertebrates, is bradycardia. Moreover, they noted that the cardiac response often mirrored, in a direct fashion, $\dot{V}O_2$ of the whole animal. Although no determination of cardiac activity was made in this study, the heart rate of another Palaemonid shrimp, Palaemon adspersus, has been reported to decrease in a linear fashion with declining oxygen concentrations (Hagerman and Uglow, 1979). Short-term exposure to petroleum hydrocarbons in solution is known to decrease heart rates in fish embryos (Anderson et al., 1977; Linden, 1978). If the chronic ingestion of DMN-contaminated food was having a similar effect on the heart rate of Palaemonetes pugio, one would perhaps expect decreased, not increased, $\dot{V}O_2$ in declining oxygen as was observed.

One explanation for the altered relationshp between $\dot{V}O_2$ and declining oxygen concentrations observed in shrimp ingesting DMN-contaminated food may be related to activity levels. During whole animal manometric $\dot{V}O_2$ determinations at 20°C (see Figure 3), a 15-20 min period was allowed, prior to $\dot{V}O_2$ measurements, because preliminary experiments had indicated high $\dot{V}O_2$ during this initial period. However, when determining $\dot{V}O_2$ in declining oxygen concentrations, no equilibration period was allowed. If the ingestion

of DMN-contaminated food for 32 days resulted in shrimp which are more active, then this could explain the high initial $\dot{V}O_2$ were observed and, consequently, the significantly greater slopes. Tatem (1977) has reported that Palaemonetes pugio exposed to water-soluble fractions of No. 2 fuel oil exhibits hyperactive behavior.

After the recovery period, the residual effects of DMN-contaminated food acted in some manner to enhance survival of Palaemonetes pugio to hypoxic conditions. None of the respiratory studies indicated why this might be so. However, shrimp which had ingested DMN-contaminated food did have low activities of the lysosomal enzyme acid phosphatase after exposure to low oxygen conditions (see Discussion on Physiological Indices of Stress).

The concentrations of hemolymph copper (Cu) reported here approximate those found in another shrimp Crangon vulgaris (Djangmah, 1970). Neither FT nor DMN-contaminated food alone had a significant impact on hemolymph Cu concentrations. However, grass shrimp exposed to both perturbations for 32 days did have significantly depressed concentrations.

Variations in crustacean hemolymph Cu concentrations have been reported to be affected by molting activity and nutritional status. Cu concentrations are elevated at the premolt stages and depressed immediately after ecdysis (Kerkut et al., 1961; Djangmah, 1970; Djangmah and Grove, 1970). If depressed concentrations in shrimp exposed to the FT/DMN regime were the result of an increased proportion of shrimp at the post-molt stage, one would perhaps expect

to observe a substantial number of exuviae in the aquarium. This was not observed. Blackman (1972) reported that the ingestion of crude oil did not affect molting in the shrimp Crangon crangon.

Djangmah (1970) found that starvation depressed Cu concentrations in the hemolymph of Crangon vulgaris while corresponding increases were noted in the hepatopancreas. The response was reversible when starved shrimp were fed. This led the authors to suggest that hemocyanin biosynthesis occurred in the hepatopancreas. It is well documented that crustacean hepatic tissues, as well as gut and excretory tissues, are rich in the enzymes which metabolize aromatic hydrocarbons (Neff, 1979). It is possible that the ingestion of DMN-contaminated food induced metabolic activity in the hepatopancreas which somehow disrupted normal hemocyanin synthesis. Fletcher et al. (1979) reported depressed Cu concentrations in marine fish exposed to crude oil for 6 months. Although no direct evidence was presented, these authors suggested that the exposure to oil disrupted the dietary uptake of Cu or the hormonal regulation of hemolymph Cu.

Although hemolymph Cu reflects hemocyanin concentrations, it says nothing about the protein's qualitative ability to extract oxygen from seawater and deliver it to the tissues. Factors such as salinity and acidic groups can and do affect the binding of oxygen to hemocyanin (Mangum, 1980). Vandermeulen, Hanrahan and Hemsworth (1980) reported no change in the oxygen dissociation curve or subunit dissociation of hemocyanin in the crab Cancer irroratus exposed to crude oil. However, they exposed crabs for only 1 h. The effects of

DMN-contaminated food or FT on the ability of hemocyanin to transport oxygen was not evaluated in Palaemonetes pugio due to the small volume of blood available for sampling. Significant changes in this parameter, which may have altered survival to hypoxic conditions, cannot be discounted at this point.

The effects of an acute change in temperature did not significantly affect $\dot{V}o_2$ of Palaemonetes pugio after the exposure period. This was not entirely unexpected since grass shrimp are known to exert a high degree of control over their aerobic metabolism when exposed to acute changes in temperature (McFarland and Pickens, 1965; Burton, Margrey and Richardson, 1976). However, shrimp exposed to FT had consistently depressed $\dot{V}o_2$ when acutely transferred to 15°C or 25°C. Exposure to FT has been reported to significantly depress $\dot{V}o_2$ in other marine invertebrates after an acute change in temperature (Widdows, 1976; Dame and Vernberg, 1978; Sastry, 1979). However, these investigators imposed a larger daily range of FT (8°-10°C) than used in the present study (4°C). This quantitative difference in the FT regime may explain why statistically significant differences for Palaemonetes pugio were not detected in this study.

After the recovery period, a somewhat different pattern of metabolic response to acute temperature change was observed. Shrimp from the FT regimes have depressed $\dot{V}o_2$ when exposed to 25°C but not when exposed to 15°C. This indicates that acclimation to the high temperatures of the FT regime is more persistent than to the low temperatures. Other studies have shown that acclimation to high

temperatures occurs more rapidly than acclimation to low temperatures (Feldmeth, Stone and Brown, 1974; Hutchison and Maness, 1979; Huppert and Laudien, 1980). Although no comparative information is available on the rate of acclimation in the opposite direction, i.e., from FT to stable temperatures, the present study indicates that the rate is slower for the high range of temperatures.

The respiration studies were generally unproductive in helping to identify the mechanisms by which FT and DMN-contaminated food affect survival of Palaemonetes pugio in hypoxic conditions. Altered $\dot{V}o_2$ in response to declining oxygen concentrations observed for shrimp ingesting DMN-contaminated food after the exposure period may explain, in part, why these shrimp had reduced survival to hypoxia. However, one must be careful not to equate correlation with causation. Why FT have such a significant detrimental effect on survival to hypoxia after the exposure period was not evident in any of the respiration studies.

Physiological Indices of Stress

In the present study neither FT nor DMN-contaminated food alone significantly affected the O:N ratios of Palaemonetes pugio. Only when shrimp were exposed to both perturbations simultaneously, were consistently elevated ratios observed both before and after the recovery period. For this reason, O:N ratios do not appear to be a particularly useful index for discriminating between stress due to FT and that resulting from the ingestion of DMN-contaminated food.

Palaemonetes pugio had depressed O:N ratios and increased nitrogen excretion rates in all four treatments after the recovery period. This indicates that grass shrimp were probably experiencing some degree of nutritional stress. This assumption is supported by decreased survival to the challenge of hypoxia + reduced salinity. ET_{50} values decreased from 2000-14,000 min after exposure to about 400 min after recovery. Regnault (1981) has also reported a decrease in O:N ratios in the shrimp Crangon crangon which were due to starvation. Mean O:N ratios in that study decreased from 27-54 to about 8 after 14 days of starvation. The decrease in O:N ratios was accompanied by a 50% loss in body protein. Although care was taken to insure that the caloric requirements of Palaemonetes pugio were being met, restricting the diet to a single food type (Artemia sp.) may not be adequate for long-term maintenance. This may be especially true in light of the fact that grass shrimp ingest a wide variety of food in the field (Adams and Angelovic, 1970; Nixon and Oviatt, 1973; Welsh, 1975; Bell and Coull, 1978; Nelson, 1979).

Tatem, Anderson and Neff (1976) reported decreasing resistance for Palaemonetes pugio acutely exposed to a standard reference toxicant (dodecyl sodium sulfate) the longer it was maintained in the laboratory. This, despite the fact that no increase in laboratory maintenance mortality was observed. The authors suggested that a qualitative deficiency in the maintenance diet (fish flake food) may have contributed to the increased sensitivity. A qualitative dietary deficiency may explain the differences in survival and the apparent increase in protein catabolism observed in this study after the

recovery period. Emerson (1967) reported that Artemia salina nauplii do not contain the amino acids taurine or methionine. Claus et al. (1979) also reported that Artemia salina nauplii do not contain taurine but did contain trace amounts of methionine. They also found that methionine is not detected when nauplii were starved for 48 h. Both taurine and methionine are found in Palaemonetes pugio (Roesijadi, Anderson and Giam, 1976b). Methionine has been shown to be an essential amino acid for both palaemonid and penaeid shrimp (Cowey and Forster, 1971; Shewbart, Mier and Ludwig, 1973; Miyjima, Broderick and Reimer, 1976). The apparent increase in protein catabolism in Palaemonetes pugio after the recovery period may be the result of a diet deficient in certain amino acids.

The water flux rate parameters determined for Palaemonetes pugio in this study are almost identical to those reported for Palaemonetes pugio by Roesijadi et al. (1976a). These workers found a decrease in water flux when grass shrimp were either transferred to water of a different salinity or exposed to polychlorinated biphenyls. They interpreted this observation as a generalized stress response of Palaemonetes pugio to environmental perturbation.

In the present study, shrimp from the FT/CLN regime had decreased water flux rates after both the exposure and the recovery periods relative to shrimp from the other three treatments. Such a consistent observation suggests that FT induce a basic change in the organism which is not easily reversed. Crustaceans (Lewis, 1962; Morris, 1971; Brichon, Chapelle and Zwingelstein, 1980) as well as other

poikilotherms (Prosser, 1973) adapt to long-term temperature changes, at least in part, by qualitative changes in the lipid fraction. The proportion of saturated fatty acids increase at high temperatures and decrease at low temperatures. This is usually interpreted as a means of maintaining the liquid-crystalline state of membranes. For synthetic membranes, water permeability has been shown to decrease as the degree of lipid saturation increased (Finkelstein and Cass, 1967). Although grass shrimp in the FT/CLN regime experienced equivalent times at 18°C and 22°C during the exposure period, their lipids may be more saturated than those of shrimp maintained at a constant 20°C. This is because poikilotherms exposed to FT often acclimate to a temperature somewhere between the mean and the upper range of the temperature cycle (Feldmeth et al., 1974; Hokansen et al., 1977; Hutchinson and Maness, 1979; Huppert and Laudien, 1980). FT did not cause a decreased water flux when shrimp also ingested DMN-contaminated food. Whatever mechanism is responsible for diminished water flux in shrimp from the FT/CLN regime, it either is not affected in shrimp exposed to both FT and DMN-contaminated food or is opposed by some process which acts to increase water permeability in the presence of DMN.

Water flux rates for Palaemonetes pugio increased in all four treatments after the recovery period, suggesting a common phenomenon was affecting all shrimp. A similar uniform response was observed both in O:N ratios and nitrogen excretion rates and was interpreted as reflecting a response to nutritional deficiency and an increase in protein catabolism after the recovery period. Mary and Krishnan

(1974) demonstrated that water permeability of crustacean exoskeletons is dependent on the presence of proteins in the epicuticle which are rich in tyrosine and sulphydryl groups. If the source of catabolized protein was the epicuticle, one might expect to observe some increase in water permeability.

An alternative explanation, which is also related to nutritional deficiency, is suggested by the work of Wilcox and Jeffries (1976). They found that as starvation progressed in the shrimp Crangon septemspinosa, the percent body water increased. The authors suggested that water was replacing the volume once occupied by tissues which had been catabolized. If a similar phenomenon were occurring in Palaemonetes pugio, one might expect an increase in water flux rates in response to the increase in body water. Percent body water was not determined in this study with Palaemonetes pugio.

Prior to hypoxia, the non-specific acid phosphatase activities in the hemolymph of Palaemonetes pugio approximate those reported for molluscs (Cheng and Butler, 1979; Cooper-Willis, 1979). Comparable data are not available for crustaceans. Exposure to hypoxic conditions produced elevated enzyme activities in the hemolymph of most groups of shrimp. Increased lysosomal enzyme activity, including acid phosphatase, has been related to tissue necrosis in rats subjected to hypoxic conditions (Abraham, Goldberg and Grasso, 1967; Ericsson, 1969) as well as marine molluscs (Moore, Lowe and Moore, 1979). The release of lysosomal enzymes, including acid phosphatase, has also been reported for human leukocytes subjected to low oxygen

tensions (Skosey et al., 1981). These authors presented evidence which suggested that a reduction in one or more products normally associated with aerobic metabolism was responsible for the release of lysosome enzymes.

The ingestion of DMN-contaminated food for 32 days did not affect the level of non-specific acid phosphatase activity in the hemolymph of grass shrimp. This was somewhat surprising since DMN has been shown to be a highly effective destabilizer of lysosomes in Mytilus edulis (Moore, 1979). This apparent discrepancy may be the result of interspecific differences but more probably due to the mode of action and level of contamination. Moore (1979) determined lysosome membrane stability 24 h after injecting mussels with a single dose of DMN at a very high concentration of approximately 1.6 g/ℓ.

After the exposure period, shrimp from both the FT regimes had elevated acid phosphatase activities when exposed to hypoxic conditions compared to shrimp from the stable temperature regimes. This was most likely due to the high temperatures of the FT regime since lysosomes are known to be destabilized when marine organisms are held at sublethal elevated temperatures (Bayne et al., 1976; Moore, 1976).

After the recovery period, the elevated non-specific acid phosphatase activities in the hemolymph of grass shrimp from the FT regimes, prior to hypoxia, were most probably due to tissue reorganization in response to the stable temperatures. Poikilotherms are known to alter their biochemical constituents in response to a

change in the thermal environment (Hochachka and Somero, 1973; Prosser, 1973). With exposure to hypoxia, shrimp which had ingested nothing but uncontaminated food had higher enzyme activities than those which had ingested DMN-contaminated food. The lower enzyme activities in shrimp which had ingested DMN-contaminated food may explain why this group of grass shrimp exhibited enhanced survival to hypoxia compared to shrimp which had ingested nothing but uncontaminated food. The reason for the lower enzyme activities in hemolymph of shrimp which had ingested the contaminated food is unclear. However, it is known that certain substances, for example some lipids and lipid soluble hormones (Koenig, 1969; Moore, 1976; Raz and Goldman, 1976), exert a protective or stabilizing influence on lysosome membranes.

None of the parameters evaluated in this study could be recommended for immediate use under field conditions. However, due to qualitative differences detected in some parameters, it appears that certain assays, e.g. hemolymph acid phosphatase activities, may be potentially more useful than others.

Increased O:N ratios following the recovery period indicated a possible qualitative nutritional stress resulting from the use of a single food type. This certainly has implications for long-term maintenance of marine organisms for culture or for toxicological studies. This parameter does not appear useful in identifying physiological stress resulting from FT or DMN contaminated food in the laboratory. However, the use of O:N ratios has been successful in

detecting pollution-induced physiological stress in Mytilus edulis, but only after sufficient baseline information had been generated (Widdows, Phelps and Galloway, 1981).

Reduced water flux rates were correlated with exposure to FT. However, this parameter does not appear useful as a stress index for two reasons. Reduced water flux rates were observed for shrimp exposed to FT even after the recovery period, a time when results of the challenge tests indicated that FT no longer affected survival to hypoxia. Second, when shrimp in FT also ingested DMN-contaminated food, water flux rates were not different from those of shrimp in the stable temperature regimes ingesting either clean or DMN-contaminated food.

The non-specific activity of the lysosomal enzyme acid phosphatase in the hemolymph of Palaemonetes pugio appears to hold the most potential as an index of stress. Activities in the hemolymph of shrimp correlated well with the major findings of the challenge tests. However, correlation must not be equated with causation. At this point, it cannot be said that the artificial stresses imposed in the laboratory affected lysosome stability directly or secondarily. Its range of application will depend on further testing on a broad range of organisms subjected to natural and pollution-induced perturbations under controlled conditions to help establish causality. Ultimately, any index purporting to detect biological stress must be tested in the field where natural variations in the index can be recorded and compared to pollution-induced perturbations. The baseline information

on any index must be accompanied by good analytical data on pollutant concentrations in the ecosystem. Only then can this information be formulated by scientists and potentially utilized by political decision-makers charged with managing the environment.

Dimethylnaphthalene (DMN) in Palaemonetes pugio

The accumulation of petroleum hydrocarbons through the diet has received very little attention. Neff (1979), in reviewing the environmental fate of polycyclic aromatic hydrocarbons, concluded that although aquatic crustaceans readily assimilate dietary aromatics, the release was also rapid and the potential for food-web contamination was therefore minimal. However, under field conditions, where the source of petroleum is not easily eliminated, the potential for biological harm and food-web contamination may exist. This may be especially important for crustaceans which, relative to vertebrates, have a limited ability to metabolize aromatic compounds (Malins, 1977). Such a situation has been reported for the fiddler crab Uca pugnax ingesting contaminated detritus in a heavily oiled marsh (Burns, 1976; Burns and Teal, 1979). Large reductions in the crab population were observed.

Because Palaemonetes pugio did accumulate DMN in the present study, a potential for biological harm and food-web contamination may exist. However, there are several arguments opposing this interpretation. First, most of the lethal and sublethal measurements made in this study indicate that the ingestion of DMN-contaminated food had a minimal detrimental effect on Palaemonetes pugio. This is

especially true when compared to the effects of FT. If the competitive fitness of grass shrimp was not altered, one would not expect a change in the population or community structure and function.

Another reason for suggesting that the accumulation of DMN observed in this study may not represent serious biological harm for Palaemonetes pugio relates to its feeding behavior. Grass shrimp have been reported to ingest a variety of food types (Adams and Angelovic, 1970; Welsh, 1975; Bell and Coull, 1978; Morgan, 1980). In the present study, shrimp were fed only a single food type. If a food source became contaminated in the field and was noxious to Palaemonetes pugio, it could presumably behaviorally select another food type if available. Some crustaceans can detect very low concentrations of naphthalene and DMN (Pearson and Olla, 1980; Pearson et al., 1980) and it is a reasonable assumption that Palaemonetes pugio has a similar capability. The consistently observed pattern of reduced tissue concentrations in shrimp once the contaminated food was removed indicates that DMN, if accumulated, would not remain in the tissues very long once the ingestion of uncontaminated food resumed.

The reason for the variation in the uptake/depuration pattern of DMN in Palaemonetes pugio is unknown. Variations in analytical procedures were less than the variation of DMN among shrimp. Similar unexplained variations in the uptake/depuration pattern of naphthalene homologs by marine invertebrates have been reported by other investigators (Cox et al., 1975; Fucik, Armstrong and Neff, 1977;

Sanborn and Malins, 1977; Tatem, 1977). Certainly, the use of a static exposure system instead of a flow-through seawater system, presents complications in determining what phase DMN is being accumulated by Palaemonetes pugio.

SUMMARY

1. Thirty-two days exposure to fluctuating temperatures (FT) and dimethylnaphthalene (DMN)-contaminated food diminished the ability of Palaemonetes pugio to resist hypoxia + reduced salinity. FT alone were quantitatively more stressful than DMN-contaminated food and stable temperatures. The detrimental effects of DMN-contaminated food were observed only in shrimp exposed to stable temperatures. FT obscured this effect.

2. After a 16 day recovery period with stable temperatures and uncontaminated food FT had no effect on shrimp's ability to resist hypoxia. Shrimp which had ingested DMN-contaminated food exhibited enhanced resistance to hypoxia.

3. Shrimp ingesting DMN-contaminated food for 32 days had elevated $\dot{V}o_2$ in declining oxygen concentrations.

4. After the 32 day exposure period, FT had no significant effect on any of the respiratory parameters, although shrimp exposed to FT did have consistently depressed, albeit non-significant, $\dot{V}o_2$ when acutely exposed to 15°C and 25°C. After the 16 day recovery period, shrimp from the FT regime exhibited depressed $\dot{V}o_2$ when exposed to 25°C but not to 15°C. These depressed respiratory

rates were offset by the stimulatory effect of residual DMN body burdens.

5. The ratios of oxygen consumed to nitrogen excreted were elevated in shrimp exposed to both FT and DMN-contaminated food after the exposure and recovery periods. O:N ratios were elevated in all shrimp after the 16 day recovery period.

6. Water flux rates were elevated in shrimp exposed to FT after the exposure and recovery periods. This was not observed when shrimp also ingested DMN-contaminated food. Water flux rates were elevated in all shrimp after the 16 day recovery period.

7. The non-specific acid phosphatase activity in the hemolymph of shrimp exposed to hypoxia correlated well with survival data from the challenge tests. After the exposure period, activities were significantly elevated in shrimp exposed to FT. After the recovery period, hypoxia induced elevated activities in the hemolymph of shrimp ingesting nothing but uncontaminated food.

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