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A Survey of Free Amino Acids in Copepod Populations

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A SURVEY OF FREE AMINO ACIDS IN COPEPOD POPULATIONS

by

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ABSTRACT

The distribution of free amino acids (FAA) in natural copepod populations was studied in six copepods. The species studied included Diaptomus sp., Eurytemora affinis, Acartia tonsa, Acartia clausi, Pseudocalanus minutus, and Calanus finmarchicus. All six species were collected from Rhode Island and neighboring waters at times of abundance. Collectively, they represent a chain of species populations that span a salinity gradient from fresh water (Diaptomus sp.) to the oceanic environment (Calanus finmarchicus). Each member of this chain has a higher optimum salinity for propagation.

FAA were analyzed by ion-exchange chromatography. Nineteen common FAA were present in all the species, with the exception of Eurytemora affinis, which had no free histidine. Both the fresh and brackish water copepods, Diaptomus sp. and E. affinis, were characterized by the predominance of alanine. In addition, the basic amino acids, arginine and lysine, constituted a large fraction of the total free amino acid content in the Diaptomus sp. Glycine was the most abundant FAA in the higher salinity species --- A. tonsa, A. clausi, P. minutus, and C. finmarchicus. Proline, alanine, taurine, glutamic acid, arginine, lysine, and threonine were also abundant. Collectively, these eight FAA constituted over 60% of the total FAA content in all the species studied.

Optimum salinities for the six species plotted against the respective FAA concentrations of glycine, taurine, proline, arginine, alanine, and glutamic acid resulted in a curvilinear relationship. The

optimum salinity-total FAA relationship was also curvilinear. The fresh water copepod Diaptomus sp. had the lowest total FAA levels of all the six species. Increasingly higher total FAA concentrations occurred in E. affinis, A. tonsa, A. clausi, and P. minutus, species with successively higher optimum salinities. Thus, a linear relationship between increasing optimum salinities and FAA concentrations in the corresponding species was obtained, with the highest FAA levels occurring in A. clausi and P. minutus. However, in the last species in the chain C. finmarchicus which is oceanic and has the highest optimum salinity, the total FAA level was actually less than in P. minutus and A. clausi. As a result, the relationship dropped off and became curvilinear.

The congeneric species in Narragansett Bay --- A. tonsa and A. clausi --- differed not only in their concentrations of taurine, glutamic acid, glycine, and alanine but also in their total FAA concentrations. Acartia tonsa had lower levels of these FAA (as well as lower total FAA) than A. clausi. This further supports the speculations of Jeffries (1962b) and Lance (1965) that A. tonsa is a more efficient osmoregulator than its congener.

The ecological significance of this relationship was considered in terms of: (1) adaptation of FAA levels to a specific salinity range, in accordance with the osmoregulatory demands of the particular environment; (2) the time period of adaptation to the respective environment; and (3) other variables such as food, temperature, state of maturity, starvation, pollution, and the ionic composition of the environment. The curvilinear relationship presented here remains to be verified with a controlled study on laboratory populations.

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I. INTRODUCTION

Invertebrates have higher intracellular amino acid concentrations than vertebrates. Furthermore, marine invertebrates have higher levels than related fresh water or terrestrial species (Camien et al., 1951; Duchâteau, Florkin and Jeuniaux, 1959; Simpson, Allen and Awapara, 1959). There is a linear relationship between the intracellular amino acid concentrations of marine invertebrates and the external sea water salinity (Shaw, 1958; Potts, 1958: Jeuniaux, Bricteux-Grégoire and Florkin, 1961a, 1961b, 1962; Lange, 1963, 1964; Lynch and Wood, 1966). Jeuniaux et al. (1961a, 1961b, 1962) hypothesized that euryhaline invertebrates can actively modify their intracellular amino acid levels through the process of "intracellular isosmotic regulation." Thus, free amino acids buffer the osmotic concentration of the tissues and enable the organism to adapt to salinity changes (Shaw, 1958; Florkin and Schoffeniels, 1965).

The mechanisms involved in copepod adaptation to brackish water are not known. The distribution patterns of copepods and experiments on their salinity tolerances indicate that certain copepods can adapt to wide salinity ranges (Wilson, 1932; Davis, 1944; Deevey, 1948; Grice, 1960; Cronin, Daiber and Hulbert, 1962; Marshall and Orr, 1955; Lance, 1963, 1964b). Intracellular isosmotic regulation may be one mechanism that enables copepods to adapt to an estuarine environment. Cowey and Corner (1963) showed that the marine copepod Calanus finmarchicus possesses a large free amino acid fraction. This fraction is similar in content to that of higher marine invertebrates; therefore, it may also be involved in the adaptation of Calanus to low salinities.

This study is a survey of the free amino acids in natural populations of six copepod species that normally live along a salinity gradient, extending from fresh water to oceanic environments. It has the following objectives: (1) to determine if the six copepod species have unique free amino acid distribution patterns; (2) to determine if there is a relationship between the salinity range of the species and its free amino acid concentration; (3) to determine the nature and the ecological significance of this relationship.

II. REVIEW OF LITERATURE

A. Definition of Free Amino Acids

The concept of a "free amino acid" has not been clearly defined. Several workable definitions have been proposed. Kittredge et al. (1962) define free amino acids as the alpha-amino carboxylic acids that can be readily extracted with 80 per cent ethanol, react with ninhydrin, and can be detected by paper chromatography. Soupart (1962) includes under the term "free amino acid" all the free amino acids, free amino acid derivatives, and substituted free amino acids in which the amino group is free to react with ninhydrin. Free amino acids have also been defined as free or easily extractable, small molecular-weight, ninhydrin reactive constituents (Roberts and Simonsen, 1962). On the other hand, Awapara (1962) considers free amino acids as being simply the alpha-amino carboxylic amino acids, and not all compounds that are detected with ninhydrin in paper chromatograms or in effluents from ion-exchange resin columns.

The "free amino acid pool", according to Britten and McClure (1962), is simply the total quantity of low molecular weight compounds which may be extracted from the cell under conditions which will not

degrade the macromolecules into low molecular weight subunits.

Throughout the present study, free amino acids will be defined as the alpha-amino carboxylic amino acids that are readily extracted with a methanol-chloroform-water solvent (2:1:0.8), react with ninhydrin, and can be detected by ion-exchange chromatography.

B. Analysis of Free Amino Acids

1. Extraction

A combination of boiling water, mechanical disruption, and subsequent treatment with detergents was found to be effective in liberating the free amino acid pool in bacteria (Gale and Taylor, 1947). Since then, a variety of other methods have been used. All involve the breaking of cellular barriers by mechanical, physical, or chemical means (Lindenberg and Massin, 1958).

Extractions have been carried out either in cold or boiling water (Lindenberg and Massin, 1958; Cowie, 1962). Numerous agents have been reported as effective in precipitating the water soluble protein in the extracts. They include: 5% or 8% trichloroacetic acid and 5% perchloric acid (Lindenberg and Massin, 1958); 10% trichloroacetic acid (Cowey, 1961); and 10% sulfosalicyclic acid (Lynch and Wood, 1966). Camien et al. (1951) used tungstic acid. Cowey and Corner (1963) preferred 80% ethanol. A 96% ethanol solution has also been utilized (Kermack, Lees and Wood, 1955; Raghupathiramireddy and Rao, 1963).

Very little knowledge exists as to the effect of the extraction procedure on amino acids in the cells. Cowie and McClure (1959) demonstrated the presence of two types of free amino acid pools in bacteria: (1) an internal pool; and (2) an expandable, concentrating pool. Hot water or 5% trichloroacetic acid extract both pools, whereas cold water

extracts only the expandable pool. Lindenberg and Massin (1958) showed that the heating of yeast extracts can cause the dissociation of amino acids that are loosely bound to protein by non-peptide bonds. Extraction with ethanol may free considerable quantities of peptides from certain microbial cells. These peptides are not obtained by other methods (Hancock, 1958).

The definition of a free amino acid depends on the method of extraction. It is surprising that only a few studies exist which are concerned with a comparison of the effectiveness of the different extraction procedures. Kermack et al. (1955) found that extractions with 96% (v/v) ethanol and extractions with water yielded similar amounts of nitrogenous material from lobster muscle tissue. Hancock (1958) used nine different methods to extract amino acids from Staphylococcus aureus and obtained equal yields. On the other hand, Lindenberg and Massin (1958) obtained different yields of tyrosine from yeast cells when extracted with cold trichloroacetic acid or boiling water. In an unpublished pilot study which compared four commonly employed extraction procedures, Jeffries (personal communication) found that each method yielded different amounts of amino acids from zooplankton samples. Thus, according to Holden (1962b, p. 74)

> The completeness of the extraction procedure should not be taken for granted when new organisms or unusual culture conditions are used.

2. Identification

Several methods have been used to identify free amino acids in the tissues of invertebrates. Microbiological assay, a rapid and qualitative method, has been used in the past (Camien et al., 1951). It is

specific only for certain amino acids (Awapara, 1962). A method used more extensively is that of paper chromatography. The technique involves a comparison of the chromatographic behavior of an amino acid with a reference amino acid in numerous solvent systems (Winitz, 1962). The ratio of the distance travelled by the amino acid to the distance of the solvent front (R_F) is characteristic of a given amino acid under given experimental conditions. The R_F value depends upon the experimental temperature, nature of the filter paper, and the organic solvent. The solvents used more often as the mobile organic phase have included collidine, n-butyl alcohol, n-propyl alcohol, phenol, and isobutyric acid (Fruton and Simmonds, 1953).

Ion-exchange chromatography, developed by Moore and Stein (1954a), is the most sophisticated method. Columns of polystyrene resins, bearing sulfonic acid groups, are treated with sodium ion buffers. There is an interaction between the resin and the sodium ions. The amino acids which are applied to the column are either absorbed or retarded, depending on their degree of basicity (White, Handler and Smith, 1964). The rate of movement of an amino acid depends upon the chemical nature of the amino acid and the solvent system used (Fruton and Simmonds, 1953). In the commercially available amino acid analyzers, the amino acids are mixed automatically with the ninhydrin reagent. The color is developed, and the absorbance is recorded as a change in current when the effluent passes a light beam directed on a photocell. Quantitative estimations can be made by integrating the area under each amino acid peak (White, Handler and Smith, 1964).

C. Origin and Intracellular State of the Free Amino Acids

1. Origin

The origin of free amino acids in invertebrates is still unknown. Two possibilities exist: (1) an extracellular origin, with subsequent transportation of free amino acids into the cell; and (2) an intracellular origin (Florkin and Schoffeniels, 1965). Awapara and Simpson (1967) suggest that the amino acids represent a steady state system characteristic of the tissue. This may imply both an extracellular and an intracellular origin.

It is not known to what extent the diet of the organism contributes to this steady state. According to Lynch and Wood (1966), the ultimate source of free amino acids for the organism must be either ingestive (Stevens and Schinske, 1961) or by direct absorption from the environment (Wood and Webb, 1966). Once in the cells, the concentration may be maintained either by intracellular metabolism or by transport across the cell membrane (Lynch and Wood, 1966).

From work on the isolated nerves of Eriocheir sinensis, Schoffeniels (1960) concluded that: (1) the amino acids responsible for the maintenance of osmotic pressure are of intracellular origin; (2) the process is not under hormonal control; and (3) the presence of Na or K ions, rather than the osmotic pressure per se, is responsible for the increase of intracellular amino acids. The studies of Rothstein and Tomlinson (1961) support the hypothesis of an intracellular origin. These workers showed that the free living nematode Caenorhabditis briggsae could biosynthesize the essential amino acids valine, lysine, and isoleucine from labelled glucose. By adding labelled formate to the incubating medium, they found the presence of labelled serine, glutamate, aspartate, and alanine. The process responsible for the increase of

amino acids with salinity could involve an interaction of amino acids from protein and keto acids from carbohydrate metabolism through transamination (Allen, 1961). Proof that the entire citric acid cycle is operative in an invertebrate Crassostrea virginica has been provided by Awapara and Campbell (1964). These workers also feel that tissues must possess the ability to deaminate the amino acids and to aminate their corresponding keto acids. Gilles and Schoffeniels (1964a, b) postulate the existence of five separate metabolic pathways for the synthesis of amino acids from pyruvate in the ventral chain of the lobster.

Microorganisms also contain readily extractable amino acids and have two types of amino acid pools (Holden, 1962a, b). The yeast Candida utilis can accumulate amino acids in both a concentrating pool and an internal pool. The expandable, concentrating pool contains amino acids absorbed from the medium. This pool can readily exchange with the environment, and it is sensitive to loss by osmotic shock. The internal pool contains intracellular amino acids that are not readily lost by osmotic shock (Cowie and McClure, 1959). The internal pool amino acids may arise from the endogenous formation of family head amino acids, such as glutamic or aspartic acid, in the expandable pool. By way of a permease system, the cell may concentrate amino acids from the medium in the expandable pool. Or, upon saturation of this system, amino acids from the medium may enter the cell by diffusion. Ultimately, the expandable pool amino acids enter the internal pool (Cowie, 1962). Thus, work on the free amino acid pools of microorganisms can provide support for both an intracellular and extracellular origin. Lynch and Wood (1966) have alluded to the possible existence of two similar pools in the oyster Crassostrea virginica L: (1) an inner pool composed of free amino acids

that do not change significantly over a salinity range of 3.4 to 26.7 o/oo; and (2) an osmotically sensitive pool, composed of taurine, glycine, proline, and alanine, that responds in a linear manner to salinity.

2. Intracellular State

Both the location and the intracellular condition of free amino acids in the cells are still under dispute. Holden (1962b) suggests that the pool is maintained in the cells by an intracellular membrane. He does not discard the possibility of retention by attachment to intracellular particles or polymers. Hydrogen bonding and van der Waals forces have also been suggested as possible retention mechanisms (Cowie, 1962).

The forces involved in the retention of the internal pool may differ from those responsible for the retention of the expandable pool. Lindenberg and Massin (1958) feel that the internal pool is bound to macromolecules, whereas the external pool exists in a relatively free form. In bacteria, at least, evidence exists for the association of the internal pool with cellular ribosomes (Cowie, 1962). The amino acids of the internal pool may also complex with soluble RNA, thus forming a compound that is an intermediate between free amino acids and protein (Lacks and Gros, 1959).

Roberts and Simonsen (1962) feel that intracellular amino acids may exist in a free state in the cytoplasm or nucleoplasm; or they are held by absorptive forces at the interphases or surfaces. Furthermore, they may exist as loose complexes with proteins, lipids, or nucleic acids. A relatively free existence could explain the behavior of the expandable pool in microorganisms as well as the osmotically sensitive pool of invertebrates. Complexes of intracellular amino acids and organic sub-

stances could explain the behavior of the internal pool of bacteria and the free amino acids of invertebrates that do not respond to salinity.

D. Function of the Free Amino Acids

Interest in the study of the free amino acids of invertebrates has centered on their possible function in cellular osmotic regulation. Fredericq (1904) observed that the ash content of marine invertebrate muscle tissue was one-half that of the blood. This implied an uneven distribution of inorganic ions in the tissues and the blood. He postulated the presence of small, organic molecules to maintain the osmotic concentration of the cells. Later, these organic substances were identified as free or easily extractable amino acids (Shaw, 1958; Potts, 1958; Florkin, 1961, 1962).

Simpson, Allen and Awapara (1959), in a survey of 17 invertebrates, found greater free amino acid concentrations in the marine species than in the terrestrial or fresh water species. These workers also found that the taurine concentration of molluscs varied with the environment. Taurine was absent in the fresh water species. This implied an osmoregulatory function for taurine. Camien et al. (1951) found high concentrations of glycine in both Homarus vulgaris and Maia squinado and suggested a similar function for glycine. Large amounts of free glycine and proline were also observed in H. vulgaris by Kermack et al. (1955). Robertson (1961) showed that free amino acids accounted for 40 - 50% of the osmotic concentration of the muscle tissue of Nephrops norvegicus. The most abundant amino acids were glycine, taurine, arginine, and proline. A similar large free amino acid fraction, representing 16 - 20% of the dry weight of the organism, is present in the marine copepod Calanus finmarchicus (Cowey and Corner, 1963). Awapara

(1962) has suggested that the high free amino acid levels in marine invertebrates may represent an adaptation to the marine environment.

A prerequisite for sustained life in brackish water is the ability of tissues to tolerate large changes in body fluid concentrations (Potts and Parry, 1964). Potts (1958) showed that two lamellibranchs Mytilus edulis (marine) and Anodonta cygnea (fresh water) adapted to changing osmolar concentrations in the following manner: (1) water moved in or out of the muscle fibers; and (2) the total sodium, chloride, and amino acid content decreased or increased, respectively. Lange (1963) demonstrated a linear relationship between the external sea water salinity and the free amino acid pool of Mytilus edulis. The increased total free amino acid concentration with increasing salinity was due primarily to increased taurine levels. Thus, taurine exerted a "sparing action" on essential amino acids.

The free amino acid pool of Carcinus maenas, a brackish water crab, undergoes similar changes when the organism is transferred from full strength to 40% sea water (Shaw, 1958). The intracellular amino acids, responsible for 60% of the total osmotic pressure, become greatly reduced. This illustrates that the importance of the free amino acids lies in their ability to reduce the cellular osmotic pressure as the blood is diluted. Duchâteau et al. (1959) found no changes in arginine, alanine, and aspartic acid concentrations when Carcinus was transferred from full strength to 50% sea water, but they did observe large reductions in the levels of glycine, proline, and glutamic acid. From work on the isolated nerves of Carcinus, Lewis (1952) postulated the following functions for free amino acids: (1) as anions to balance essential, internal cations; and (2) as extra substances to maintain osmotic equilibrium.

The relationship between free amino acid pools and salinity was investigated in a variety of euryhaline marine invertebrates (Jeuniaux et al., 1961a, b; 1962). Organisms adapted to fresh water had lower free amino acid levels than organisms adapted to sea water. The greatest variations in concentration were observed for glycine, alanine, proline, glutamic acid, and taurine. The hydration of the tissues was not great enough to explain these variations. An active modification of the intracellular amino acids ("intracellular isosmotic regulation") was proposed as a mechanism of adaptation to salinity changes.

Lynch and Wood (1966) also suggested that the free amino acids of Crassostrea virginica function in maintaining an osmotic equilibrium with the environment. They found that the total free amino acid concentration increased proportionately with increasing salinity, over a range of 3.4 - 26.7 o/oo. Taurine, glycine, proline, and alanine were responsible for most of the increase. The possibility that two types of free amino acid pools could exist in invertebrates was implied by the observation that a portion of the free amino acid pool did not vary significantly with salinity. This portion included the amino acids: cysteic acid, aspartic acid, threonine, serine, cystine, tyrosine, phenylalanine, ornithine, lysine, and arginine. All were present in concentrations less than 0.25 µmoles/mg of total Kjeldahl nitrogen.

In Rangia cuneata, alanine shows the greatest change with salinity. But, in this case, the free amino acid pool, as typified by aspartic acid, glutamic acid, glycine and alanine, increases in a curvilinear manner with increasing salinity. Up to a salinity of 17 o/oo, the concentrations of all four amino acids increase in a linear manner. At salinities of 20 and 25 o/oo, all concentrations are markedly decreased (Allen,

1961). The author suggests that a shift in osmotic control at 17 o/oo is responsible for the observed decrease at the higher salinities.

Small changes in salinity may have an important effect on free amino acid levels. Virkar (1965, 1966) showed that the free amino acid pool of the sipunculid Golfingia gouldii is far more sensitive to a 10% decrease in salinity than it is to a large dilution of the external medium. This suggests that the mechanism of intracellular isosmotic regulation may operate more efficiently with modest decreases in salinity. At large dilutions, the organism may be merely able to survive. In estuaries, organisms are subjected daily to small variations in salinity (Lockwood, 1962); hence, the response of the free amino acid pool to small changes becomes important.

Based on all the available knowledge, Florkin and Schoffeniels (1965) have postulated two mechanisms for the survival of organisms in an estuarine environment: (1) anisosmotic extracellular regulation, and (2) intracellular isosmotic regulation. All marine invertebrates are believed to possess the latter mechanism. The former mechanism, if present, can greatly extend the euryhalinity of a species by relieving the intracellular mechanism of part of its task. The importance of free amino acids lies in the intracellular isosmotic regulation.

In microorganisms, the free amino acid pool may have other functions. Hancock (1960) observed an accumulation of most pool amino acids when protein synthesis was blocked in Staphylococcus aureus. The rate of accumulation of glutamic acid, aspartic acid, and proline equalled their rate of incorporation into protein. This implied that an important function of internal pool amino acids was to provide precursor material for protein synthesis. Based on their work on Escherichia coli, Lacks

and Gros (1959) concluded that the soluble RNA-amino acid complexes, formed by a small portion of the internal amino acid pool, served as intermediates in protein synthesis. The internal pool of Sarcina lutea was shown to act as the natural reserve for endogenous metabolism (Dawes and Holms, 1958).

E. Regulation of the Free Amino Acid Pool

Marine invertebrates adapt to the estuarine environment because they possess the ability to control their tissue amino acid concentrations (Florkin and Schoffeniels, 1965). The mechanism that regulates the free amino acid levels in response to salinity is not known.

Kavanau (1953) found high concentrations of both free glycine and peptide glycine in the sea urchin embryo. He proposed the presence of a dynamic equilibrium between the osmotically active free glycine and the inactive peptide glycine to explain the buffering action of glycine in the cells. But his results did not substantiate this. While the peptide-glycine fraction decreased, no substantial increase occurred in the free glycine levels. Shaw (1958) showed that the process responsible for the decrease of free amino acids in Carcinus maenas at reduced salinities was reversible. This implied that the amino acids were not removed from the muscle fibers. Instead, they probably combined with the protein molecules of the cell.

Extensive studies by Schoffeniels and Gilles (1963) on the enzyme L-glutamic acid dehydrogenase of Astacus fluviatilis demonstrated that the enzyme required both Na and K ions for maximum activity. If the essential step in osmoregulation is a balance between the degradation and synthesis of amino acids, the cations could affect the process by activating or inhibiting enzymes related to amino acid metabolism. In steno-

haline organisms, pool levels would be kept constant by a simultaneous stimulation of both the degradation and synthesis mechanism. But in euryhaline invertebrates an independent control over the mechanisms of catabolism and anabolism of amino acids would allow the organisms to regulate their free amino acid concentrations (Gilles and Schoffeniels, 1964b).

The work of Yunis, Arimura and Kipnis (1963) lends support to the theory of cationic regulation of free amino acid pools. The authors showed that both Na and K ions were needed for the maintenance of maximum rates of amino acid transport in human leukocytes. According to Allen (1961), free amino acid levels could be controlled by transamination reactions between free amino acids and short chain keto acids which arise from carbohydrate metabolism. Transaminases have been found in the invertebrates Crassostrea virginica (Awapara and Campbell, 1964) and Carcinus maenas (Chaplin, Higgins and Munday, 1967). A change in the Na/K ration of estuarine waters could also control the free amino acid levels of organisms (Lynch and Wood, 1966).

Lange (1963), on the other hand, feels that free amino acid levels of Strongylocentrotus drobachiensis are controlled by changes in cell permeability. He does not discard the possibility of entry into biochemical reactions as a means of control. According to Stephens and Virkar (1966), a salinity stress controls pool levels by stimulating the rates of entry and exit of amino acids from the pool. A decrease in salinity stimulates the rate of incorporation of free amino acids into polypeptides, thus decreasing pool size and reducing the internal osmotic pressure.

F. Distribution of Copepod Species

1. Diaptomus sp.

This genus has been little investigated in the New England area. Its members are only fresh water in distribution (Wilson, 1932).

2. Eurytemora affinis (Poppe)

Jeffries (1967) classifies this species as "true estuarine" on the basis that its propagation is limited to brackish water. The optimum range for reproduction is 5 - 15 o/oo. The studies of Deevey (1948) have, likewise, shown that this is a true estuarine organism. She did not find it in Long Island Sound where salinities varied from 24 - 29 o/oo. Cronin et al. (1962) found abundant populations of this species in the upper Delaware River estuary at salinities from 1 - 10 o/oo. Above 20 o/oo, it disappeared. According to Wilson (1932), this species is not confined to salt water but can ascend estuaries into fresh water. The presence of a reproducing population has been reported in Lake Erie (Engel, 1962).

The abundance of E. affinis in any environment depends on two factors: (1) distance from fresh water, and (2) the portion of the length of the estuary that is encompassed by the 5 - 15 o/oo isohalines (Jeffries, 1962a). The maximum abundances occur, therefore, in the 5 - 15 o/oo salinity range. All the representatives of this genus appear to be boreal forms, abundant in local estuaries in winter and spring (Jeffries, 1962a).

3. Acartia tonsa (Dana)

Acartia tonsa is the dominant summer-fall copepod of Narragansett Bay (Jeffries, 1962b,1967). This species is widespread in warm, shallow estuaries (Lance, 1963), and, in general, it thrives in low salinity

waters (Wilson, 1932; Deevey, 1948; Conover, 1957: Jeffries, 1962b).

The following salinity ranges have been reported for this species: 0.7-34.3 o/oo (Davis, 1944); 5.2-29.8 o/oo (Deevey, 1948); 24.4-38.4 o/oo (Grice, 1960). Wilson (1932) has reported it in fresh water. Cronin et al. (1962) found maximum populations of this species in salinities from 5 - 20 o/oo. Large endemic populations also occurred in 20 - 30 o/oo salinities.

Of all the Acartia species, A. tonsa is the most tolerant to dilution (Lance, 1963; Jeffries, 1962b). But salinities lower than 7.4 o/oo (or 20% sea water) do cause mortality in the laboratory (Lance, 1963). Lance (1964a,1965) showed an increased oxygen consumption and a decreased grazing activity for this species at low salinities. This suggested the possibility that A. tonsa had difficulty in maintaining itself in the estuarine environment. Its widespread distribution and abundance in estuaries did not support this conclusion. Hence, she suggested that A. tonsa adapted to salinity fluctuations in nature. The increased oxygen consumption at low salinities may indicate the degree of hydration of the tissues (Schlieper, 1936).

4. Acartia clausi (Giesebrecht)

Acartia clausi dominates the winter-spring plankton of Narragansett Bay (Jeffries, 1962b). This species has a world-wide distribution in coastal estuaries (Wilson, 1932), but Sewell (1948) feels that it may be more successful in coastal waters. Bigelow and Sears (1939) showed A. clausi to be confined to coastal waters south of Cape Cod, but to the north it could occur offshore as well.

This species has been reported in low salinity waters (Gurney, 1931; Wells, 1938; Conover, 1957; Raymont and Carrie, 1958, 1959). Of

the four Acartia species studied by Lance (1964b), this was the least tolerant to dilution. Deevey (1948) reported a salinity range of 9-29.9 o/oo for this species. Lance (1964b) showed that the salinity range that caused mortality in the laboratory varied from 0 - 45% sea water (or 0-15.8 o/oo) for an adult female and from 0 - 55% sea water (or 0-19.2 o/oo) for an adult male.

Martin (1964) feels that A. clausi shows no definite salinity preference: salinity is less significant in determining its abundance than temperature.

Both A. clausi and A. tonsa are "estuarine and marine" organisms. They can propagate in estuaries, but population development is limited by a 10 o/oo salinity (Jeffries, 1967).

5. Pseudocalanus minutus (Krøyer)

P. minutus is a northern species, widely distributed in the North Atlantic region (Bigelow, 1926). It can occur in the Gulf of Maine throughout the year; however, it has been reported to disappear from the neritic zone south of Cape Cod during the summer months (Fish, 1936).

A salinity range of 7.25 - 35.3 o/oo has been reported for this species (Deevey, 1948). Although it can maintain itself in low salinity waters, it is primarily a neritic species. Cronin et al. (1962) found it able to penetrate the Delaware River estuary to a salinity of 9.6 o/oo in the winter and to 8.3 o/oo in the summer. In the Woods Hole area, the greatest populations and numbers occur in salinities of 30 - 32 o/oo (Anraku, 1961). Faber (1959) also found more abundant populations of this species in the colder and more saline waters of the mouth of Narragansett Bay than in the estuarine areas, such as the West Passage of the Bay.

According to Jeffries (1967), this is a "euryhaline marine" species. To propagate in an estuary, a population must rely on continuous recruitment from the ocean.

6. Calanus finmarchicus (Gunnerus)

This is primarily a pelagic species. It is widely distributed in all the oceans, including the Arctic and Antarctic, and along the Atlantic and Pacific coasts of America. However, it can occur in Narragansett Bay, Buzzards Bay and Vineyard Sound in the winter and early spring (Wilson, 1932). Deevey (1952) found the greatest numbers of Calanus in Block Island Sound during spring and summer. There it was primarily neritic. She also found it in Long Island Sound. Martin (1964) reported small numbers of this species at the mouth of the West Passage of Narragansett Bay during both winter and summer months.

Sewell (1948) reports a salinity range of 29-35.3 o/oo for this species. Marshall and Orr (1955), however, have stated that Calanus can successfully adapt to salinities as low as 27 o/oo and survive in sea water of 12 - 17 o/oo in the laboratory. According to Jeffries (1967), this is strictly a "stenohaline marine" species, characteristic of open, neritic waters.

III. THE INVESTIGATION

A. Apparatus and Methods of Procedure

1. Sampling Procedure

Copepod samples were taken during the period 1965-1967. All samples were collected with a number two mesh net, 0.5 meters in diameter, either by oblique or horizontal hauls through the water column. The *Calanus* sample was taken with a number two mesh net, one meter in diameter.

Six copepod species were collected from areas that represent a natural salinity gradient, extending from fresh water to the open ocean. Seasonal hydrological surveys of the respective areas indicate that the salinity gradient is a real environmental gradient (Horton, 1958; Hicks, 1959). Fresh water copepods of the genus *Diaptomus* were obtained from Wordens Pond, Rhode Island, in August, 1967. Samples of the brackish water copepod *Eurytemora affinis* were taken from Pettaquamscutt River, at the Bridgetown Bridge, during February - March, 1967. The estuarine species *Acartia tonsa* and *Acartia clausi* were collected at Station I (41°34'13"N, 71°23'30"W) during October, 1965 - October, 1966. This station is located in the West Passage of Narragansett Bay, five miles from the mouth of the Bay. The samples of *Pseudocalanus minutus* were taken at Station II (41°20'30"N, 71°20'00"W) in Rhode Island Sound during December - January, 1965 - 1966. Station II is located seven miles offshore from the mouth of the Bay. The marine copepod *Calanus finmarchicus* was obtained 10 miles north of Provincetown, Massachusetts on August, 1967.

The data for the P. minutus, A. clausi and two of the A. tonsa samples were provided by H. P. Jeffries. The C. finmarchicus sample was provided by the Bureau of Commercial Fisheries at Woods Hole.

Surface temperature and salinity were recorded at each station.

2. Handling of Samples

Immediately upon collection, the samples were placed in wide-mouthed glass jars with modified gauze-screen covers. The containers were inverted, and water drained from the samples by removing electric tape from a small opening at the bottom of the container. The jars were stored in a portable refrigerator until brought to the laboratory.

In the laboratory, the samples were rinsed with distilled water, drained, and transferred to preweighed 100 ml glass serum bottles. The samples were then freeze dried, or they were held at -20°C until freeze drying could be done. After freeze drying, the serum bottles were reweighed to determine dry weights. The dry contents were transferred to vials which were stored in a vacuum dessicator at -20°C until analysis of the free amino acid content could be carried out. This was usually done within a week.

The C. finmarchicus sample was stored in paper ice cream containers at -10°C. The containers were transported from Woods Hole to the Narragansett Marine Laboratory in an insulated box, filled with dry ice. No thawing of the sample occurred during the transportation and subsequent storage. The following day, portions of the sample were freeze dried and the dry contents stored at -20°C. Not more than a week elapsed between the period of sampling, storage, and freeze drying.

3. Extraction Procedure

Free amino acids and lipids were extracted from the whole organism by using a method based on that of Bligh and Dyer (1959). Approximately 0.5 g dry weight of the copepod sample was homogenized with 30 ml of a chloroform-methanol-water solvent (2:1:0.8). An additional 8 ml of chloroform and 8 ml of distilled water were added. Each addition was followed by a one-minute homogenization. Total homogenization time at 5-6000 RPM was seven minutes.

The resulting homogenate was divided equally between two 40 ml plastic centrifuge tubes and centrifuged at 10,000 RPM for 10 minutes. The contents were vacuum filtered through a glass fiber filter into a 50 ml separatory funnel. At this step, the insoluble proteins were removed. The epiphase contained water soluble free amino acids; the hypophase contained lipids. The two phases were separated and evaporated to dryness at 60°C. The epiphase residue was resuspended in 10% isopropanol and stored in the refrigerator until analysis. The hypophase residue was dissolved in petroleum ether which was evaporated with a stream of nitrogen gas. The lipids were weighed and their concentration expressed either in weight per cent of the tissue extracted or in mg.

4. Nitrogen Content of the Extract

A modified ninhydrin-colorimetric method (Moore and Stein, 1954b) was used to determine total nitrogen concentrations in the samples prior to ion-exchange analysis. Previous work in this laboratory showed that a concentration range of 50 - 95 μmoles alpha nitrogen/ml of extract obtained the maximum resolution from the amino acid analyzer.

Ten μliters of the extract were combined with one ml of distilled water and one ml of ninhydrin reagent. The solution was mixed, covered,

and placed in a boiling water bath for 15 minutes. A distilled water blank was prepared in a similar manner. After removal from the heat, 5 ml of 50% ethanol were added to each tube. The resulting solution was mixed and left to stand for 15 minutes in the dark. The optical density was read on a spectrophotometer (Bausch and Lomb, Spectronic 505) at 570 mμ. A standard curve, prepared with a mixture of 18 amino acids, was used to determine the approximate concentration of ninhydrin reactive substances. The total alpha-amino nitrogen content of the samples was adjusted to lie within the range of 50 - 95 μmoles/ml by either dilution with 10% isopropanol or by concentration through evaporation.

5. Ion-Exchange Analysis

The Technicon Amino Acid Analyzer, model NC-1(Ardsley, N. Y.), was used to identify and quantify the free amino acid content of the samples.

Samples for the analyzer were prepared by combining 0.4 ml of the isopropanol extract, 0.4 ml of a 2.5 μmoles/ml norleucine solution, and 0.2 ml of a 62.5% sucrose solution. Subsequently, 0.2 ml of this solution was injected with a microsyringe onto the top of a 130 x 0.6 cm column, packed with Technicon's Type B resin. The temperature of the column was maintained at 60°C.

Each of the nine interconnected chambers of the autograd was filled with 75 ml of sodium acetate buffers, representing a smooth gradient of pH from 2.875 - 3.800 - 5.000. Methanol was added to the first two chambers. The buffer gradient was pumped through the column at a rate of 30 ml/hr (or 0.5 ml/min).

The column effluent was mixed with a dilute ninhydrin reagent and pre-purified nitrogen gas. The color was developed in a heating

bath set at 95°C. The effluent was then pumped through a triple colorimeter assembly, and the light absorbance at 440 and 570 mμ was plotted on a logarithmic scale by a three channel recorder. The entire operation involved 18 hours running time.

6. Quantification

Quantitative free amino acid values were obtained by integrating the areas under the amino acid peaks.

The net height of the peak, in optical density units, was obtained by subtracting the base line from the gross height of the peak. The half height of the peak was obtained by adding the base line height to one-half of the net height. To determine the width of the peak, all dots above the half-height line were counted (one dot/12 sec). The distances between the first and last dots and the half-height line were estimated and added onto the value obtained by counting all the dots above the half-height line.

The net height times the width represents the area of the peak. The areas were corrected with respect to the response of the internal norleucine standard. The corrected areas were referred to standard areas obtained with a solution of 18 amino acids. To this standard solution (obtained from the Technicon Corp.) were added taurine and norleucine (2.5 umoles/ml). The concentration of each amino acid, in μmoles/g dry weight, was then computed.

7. Statistical Analysis

To determine the reproducibility of the extraction procedure, a single sample of Acartia tonsa was split into six equal portions of 0.5 g dry weight. The six sub-samples were extracted under uniform conditions by using the method described previously. The six extracts were analyzed

successively on the amino acid analyzer. The appropriate statistics, based on Snedecor (1956), were computed for five of the extracts; one sample was lost.

The precision of the ion-exchange apparatus was tested by successive analyses of four amino acid standards.

8. Salinity Determinations

Salinity was analyzed by an induction salinometer (Industrial Instruments, Model RS5-3) or by silver nitrate titration (Barnes, 1959).

B. Results

1. Variation in Total Free Amino Acids

Total free amino acid concentrations of individual samples are listed for the six copepod species in Table 1. Also listed in Table 1 are the number of samples analyzed, environmental data, and the per cent of the total catch represented by the species (% abundance).

In general, not enough planktonic material was available for replicate free amino acid determinations. However, replicate analyses were obtained where possible. The last column of Table 1 lists the number of replicates of each sample. In this case, the total free amino acids represent an average value. Cowey and Corner (1963), using 80% ethanol to extract the free amino acids and precipitate the protein, previously carried out an analysis of the free amino acid content of Calanus finmarchicus. Their value is listed here. The value of 350.5 µmoles/g dry weight for C. finmarchicus from the Atlantic Ocean represents the average total free amino acid concentration of triplicate analyses on a single sample, extracted with a chloroform-methanol-water solvent.

Duplicate analyses of the free amino acid content of a single sample of Diaptomus yielded values of 74-88 µmoles/g dry weight (Table 8,

Table 1. Environmental data, % abundance, and total free amino acid concentrations of copepod species from Wordens Pond, Pettaquamscutt River, Narragansett Bay, Rhode Island Sound and the Atlantic Ocean (10 miles north of Provincetown, Massachusetts). Total free amino acids are expressed as μmoles/g dry weight. Pett. R. (Pettaquamscutt River), Nar. B. (Narragansett Bay), RIS (Rhode Island Sound). October, 1965 - August, 1967

Species	Month	Location	Surface Salinity ‰	Surface Temperature C°	Abundance %	Total Free Amino Acids μmoles/g	Replicates
Diaptomus	Aug. 9	Wordens Pond	Fresh Water	25.7	80.0	80.2	2
Eurytemora affinis	Feb. 9	Pett. R.	19.30	1.0	90.0	217.6	1
	Mar. 8		5.90	2.0	98.0	201.9	2
Acartia tonsa	Oct. 6*	Nar. B.	31.90	13.9	99.7	650.0	1
	Nov. 3*		31.92	10.4	96.2	295.8	1
	Aug. 17		31.83	20.6	99.6	340.7	1
	Oct. 6		31.85	15.5	99.7	399.8	1
	Oct. 11		31.69	15.5	96.2	352.1	2
	Oct. 18		31.80	14.0	98.1	544.2	5
Acartia clausi	Dec. 1*	Nar. B.	31.54	6.4	90.2	695.8	1
	Dec. 29*		31.31	3.0	81.5	614.9	1
	Apr. 20*		30.37	6.3	82.1	595.3	1
Pseudocalanus minutus	Dec. 29*	RIS	32.27	6.3	89.0	687.3	1
	Jan. 26*		32.16	2.4	82.8	420.9	1
Calanus finmarchicus	Aug. 17	Atlantic Ocean	31-32	----	94.7	350.5	3
	May	Firth of Clyde[a] England	-----	----	100.0	487.1	1

*Data provided by H. P. Jeffries.

[a]Cowey and Corner (1963). Wet weight values converted to μmoles/g dry weight.

Appendix II). Only the mean value is listed in Table 1. Total levels in this species are considerably lower than the total values obtained for the estuarine and marine copepods.

The two Eurytemora affinis samples had total free amino acid values of 202-218 μmoles/g dry weight. The lower value occurred in the sample collected on March 8, at a salinity of 5.9 o/oo. This lower value represents an average of two replicate free amino acid determinations.

In the six samples of Acartia tonsa, total free amino acid concentrations varied from 296-650 μmoles/g dry weight. The highest value occurred in the sample obtained in October, 1965; the lowest occurred in the sample obtained in November, 1965. Total free amino acids of the samples collected during 1966 varied from 341-544 μmoles/g dry weight. With the exception of the 544 value, representing the average of five replicates, the agreement in total values of the other three samples was relatively good.

The total free amino acid content of the three Acartia clausi samples varied from 596-695 μmoles/g dry weight. The lowest value occurred in the April, 1966, sample; the higher values occurred in the samples collected during December, 1965. Both the Acartia species were obtained at Station I in Narragansett Bay but at different times of the year: A. tonsa during August-November; and A. clausi during December-April. The salinity during this period varied only from 30.37-31.92 o/oo. However, temperature varied from 3.0-20.6°C.

Two Pseudocalanus minutus samples, collected from Rhode Island Sound during the winter of 1965-1966, had total free amino acid values of 421-687 μmoles/g dry weight. The higher value occurred in the December 1965 samples; the lower in the sample collected in January, 1966.

The total free amino acid concentrations of the six copepod species varied considerably not only among the species but also within samples of the same species.

Table 1 further shows that, at the time of sampling, the six copepod species were the dominant members of plankton communities (% abundance) along sectors of a salinity gradient that extends from fresh water to the open ocean. Wordens Pond is strictly fresh water. Low salinity occurs in Pettaquamscutt River which, during the period of sampling, had surface salinity values of 5.9-19.3 o/oo, with a mean of 12.6 o/oo. The two salinity measurements at Bridgetown Bridge are characteristic of the surface salinity of the upper river (Table 15, Appendix II). Horton (1958) showed that surface salinity in the Lower Pond of the river varied from 11.13 o/oo (Jan. 28) to 18.91 o/oo (Oct. 15). In the Upper Pond, surface salinity varied from 2.99 o/oo (Dec. 17) to 17.55 o/oo (Oct. 15). In the area of Pettaquamscutt River that was sampled, salinity fluctuated widely but the salinity was always lower than in Narragansett Bay (Tables 14 and 15, Appendix II).

The surface salinity of Narragansett Bay varied only from 30-32 o/oo, with a mean of 31.44 o/oo, during 1965-1966 (Table 14, Appendix II). During the same period, the surface salinity of Rhode Island Sound varied from 31-33 o/oo, with a mean of 32.22 o/oo. Although the salinity difference between Pettaquamscutt River and Narragansett Bay is great, a smaller yet nevertheless real salinity difference persists between Narragansett Bay, Rhode Island Sound, and the offshore waters of the Atlantic Ocean.

2. Reliability of the Results

The results of the analysis to determine the precision of the analytical procedure appear in Table 2. The averages of five replicate

Table 2. Precision statistics calculated from the results of five replicate analyses on a single sample of Acartia tonsa, obtained from Narragansett Bay, October 18, 1966. Mean and range values expressed as μmoles/g dry weight.

AMINO ACID	Mean μmoles/g	Range μmoles/g	Standard[a] Deviation S_D	Coefficient of[b] Variation (CV) %
CYS	1.8	1.7 - 2.1	0.2	8.7
TAU	53.6	41.8 - 70.5	6.6	12.3
ASP	6.9	6.3 - 7.7	0.5	7.7
THR	16.7	15.6 - 18.1	1.0	5.7
SER	15.3	14.5 - 15.9	0.6	3.7
GLU	18.1	16.9 - 20.3	1.3	7.4
PRO	109.4	85.6 - 153.0	31.1	28.4
GLY	117.1	97.2 - 160.7	26.4	22.6
ALA	59.6	53.4 - 71.6	7.5	12.6
VAL	13.7	12.2 - 16.5	1.8	13.5
METH	7.1	6.0 - 9.1	1.3	18.2
ILEU	10.1	8.1 - 12.7	1.9	18.6
LEU	18.7	16.4 - 22.2	2.3	12.4
TYR	6.9	5.5 - 8.8	1.4	20.6
PHE	7.5	5.7 - 9.8	1.7	22.0
ORN	0.8	0.5 - 1.0	0.2	29.1
LYS	26.2	23.3 - 30.0	2.5	9.4
HIS	4.5	3.4 - 5.9	0.9	20.1
ARG	50.2	42.9 - 64.4	9.3	18.5
TOTAL	544.2	474.2 - 698.3		
N*	5			

*Number of replicates.

[a] S_D =

[b] CV =

analyses on a single sample of Acartia tonsa are listed in the first column. Other columns list the range of the five individual determinations, the standard deviation, and the coefficient of variation (CV) for each amino acid.

The coefficient of variation ranges from 4% for serine to 29% for ornithine. It represents the error over the entire analytical procedure, from sampling to extraction and analysis. Large coefficients of variation were obtained for both proline (28%) and glycine (23%). This observation indicated that the poor reproducibility of these two amino acids could have resulted from an error introduced into the extraction procedure, possibly during the evaporation phase. As a result, the coefficients of variation were determined for every replicate analyses. A mean of all coefficients of variation was obtained and, from this, the final error was estimated (Schilling, 1966; Wildeman, 1966). The results are listed in Table 3. Generally, the final estimate of error approximates the mean coefficient of variation. In the case of amino acids that were present in small amounts, such as ornithine, greater errors were assigned.

The reproducibility of the methionine, histidine, and ornithine determinations is relatively poor. Since these amino acids are present in small amounts (small, broad peaks), part of the error may result from recording and integration of these peaks. Large errors were also obtained for tyrosine (17%) and phenylalanine (20%). At times, these amino acids are poorly resolved which may partly explain their large error. The 17% error for aspartic acid may be due to the breakdown of asparagine or the elution of methionine sulphone with aspartic acid (London, 1966). Apart from these amino acids, the majority of amino acids have an error

Table 3. Precision statistics calculated from the results of two replicate analyses on a sample of Diaptomus sp., of Eurytemora affinis, and of Acartia tonsa, five replicate analyses on a sample of A. tonsa five replicate analyses on a sample of Calanus finmarchicus. Values represent the coefficient of variation, CV and are expressed in per cents (%).

AMINO ACID	Diaptomus CV[1] in %	Eurytemora affinis CV in %	Acartia tonsa CV in %	Acartia tonsa CV in %	Calanus finmarchicus CV in %	Average Coefficient of Variation, % CV	Final Estimate[a] of Error, % CV
CYS	12.9	5.3	33.5	8.7	4.0	12.9	13
TAU	20.1	1.3	5.0	12.3	11.6	10.1	12
ASP	28.5	11.7	18.9	7.7	17.8	16.9	17
THR	8.2	13.7	20.6	5.7	19.4	13.5	14
SER	11.9	17.2	4.0	3.7	22.7	11.9	12
GLU	7.2	12.6	4.6	7.4	16.8	9.7	11
PRO	10.8	13.6	4.6	28.4	10.4	13.6	13
GLY	13.9	5.3	0.9	22.6	6.8	9.9	11
ALA	10.9	11.1	5.1	12.6	18.2	11.6	11
VAL	11.7	24.9	13.9	13.5	24.6	17.7	15
METH	22.5	17.7	15.7	18.2	14.1	17.6	18
ILEU	9.6	21.1	12.6	18.6	14.0	15.2	15
LEU	10.1	21.0	8.2	12.4	16.4	13.6	13
TYR	6.9	31.5	13.2	20.6	13.1	17.1	17
PHE	20.3	36.1	12.8	22.0	14.4	21.1	20
ORN	2.0	8.8	----	29.1	----	13.3	20
LYS	12.9	14.1	7.9	9.4	9.7	10.8	11
HIS	16.1	----	24.6	20.1	----	20.3	20
ARG	13.3	6.5	3.6	18.5	----	10.5	12
N*	2	2	2	5	3		

*Number of replicates

[1]CV is the coefficient of variation

[a]Represents an assigned value that is representative of the overall error. It is based on individual coefficients of variation, the average coefficient of variation, and a consideration of the shape of the amino acid peak.

of 10-15% with the chloroform-methanol-water extraction method. The reduced errors of proline and glycine (13% and 11%, respectively) indicate that the coefficients of variation for the five replicates, which were unusually high for these particular amino acids (Table 2), are questionable. Possible sources of the final error include: (1) pipetting; (2) evaporation and transfer of solutions; (3) poor resolution of some amino acids; (4) integration.

The results of the experiment to determine the reproducibility of the ion-exchange apparatus are listed in Table 4. Mean areas of 19 amino acids, standardized with respect to the response of the internal standard (2.5 μmoles/ml norleucine) are listed in column 1. Also listed are the standard deviation, coefficient of variation, and the number of replicates on which the means are based. The reproducibility of the ion-exchange apparatus for the majority of amino acids is approximately 5%.

3. The Absolute Distribution of Free Amino Acids in Six Copepod Species

Mean values, in μmoles/g dry weight, of 18 free amino acids are listed for the six copepod species in Table 5. Also shown in Table 5 are the number of samples or replicates from which the average was compiled (N,N*) and the mean total concentrations, along with their standard deviations. Free amino acid concentrations of individual samples are listed in Tables 8-13, in Appendix II.

Figure 1 shows a schematic representation of the absolute distribution of these 18 free amino acids, expressed as a function of the salinity regime that each species best characterizes. For purposes of illustration, species I-VI are listed on the horizontal axis, according to increasing optimum salinities, as described previously for each species,

Table 4. Precision statistics calculated from the results of replicate analyses on four Technicon amino acid standards consisting of 18 amino acids, plus taurine and norleucine at 0.2 uM concentrations.

AMINO ACID	Mean Area (relative to norleucine)	Standard Deviation S_D	Coefficient of Variation $(CV)_1\%$
CYS	0.833	0.045	5.36
TAU	0.668	0.026	3.94
ASP	0.755	0.075	9.90
THR	0.747	0.035	4.63
SER	0.765	0.036	4.70
GLU	0.731	0.041	5.63
PRO	0.223	0.010	4.47
GLY	0.822	0.033	4.02
ALA	0.775	0.039	4.99
VAL	0.766	0.049	6.12
METH	0.779	0.047	4.05
ILEU	0.757	0.032	4.75
LEU	0.800	0.037	4.67
TYR	0.767	0.036	4.69
PHE	0.764	0.040	5.23
LYS	0.878	0.035	3.93
HIS	0.863	0.039	4.48
ARG	0.781	0.020	2.56
N*	4		

*Number of replicates.

Table 5. Mean free amino acid concentrations of copepod species from Wordens Pond, Pettaquamscutt River, Narragansett Bay, Rhode Island Sound and the Atlantic Ocean (10 miles north of Provincetown, Massachusetts). N is the number of samples analyzed. All values expressed in μmoles/g dry weight. 1965-1967. s_D is the standard deviation.

AMINO ACID	Diaptomus μmoles/g	Eurytemora affinis μmoles/g	Acartia tonsa μmoles/g	Acartia clausi μmoles/g	Pseudocalanus minutus μmoles/g	Calanus finmarchicus μmoles/g
CYS	1.2	2.1	2.0	3.3	2.1	3.3
TAU	3.0	29.3	49.7	86.4	83.7	35.6
ASP	2.4	2.2	4.0	5.8	3.8	8.7
THR	5.5	5.8	9.6	13.6	12.3	13.0
SER	4.0	6.9	9.3	10.7	8.8	11.3
GLU	4.7	12.9	12.6	17.0	10.1	6.5
PRO	3.2	27.8	78.7	98.6	109.4	22.3
GLY	4.6	20.0	118.2	196.0	187.0	77.8
ALA	12.4	41.1	45.4	65.8	40.9	36.2
VAL	3.9	5.3	10.3	14.5	10.6	15.6
METH	1.3	3.0	4.4	5.5	4.0	6.2
ILEU	2.5	3.9	6.4	9.4	6.9	10.9
LEU	4.7	6.3	11.6	15.8	12.1	19.0
TYR	2.9	3.4	4.7	7.1	6.3	8.1
PHE	2.2	3.9	5.0	7.9	5.1	7.4
ORN	0.5	1.3	+	+	+	1.2
LYS	7.1	8.6	15.1	18.4	14.5	16.3
HIS	2.0	------	2.9	4.3	4.0	3.6
ARG	12.1	26.2	41.0	55.3	32.2	47.5
TOTAL	80.2	210.0	430.9	635.4	553.8	350.5
N	1	2	6	3	2	1
s_D	10.1	15.0	128.1	43.6	133.3	19.6
N_1*	2	3	11	3	2	3

*The number of determinations, including replicates.

from fresh water (Diaptomus, I) to offshore sea water (Calanus finmarchicus, VI). This differs from a laboratory situation where one species is subjected to a succession of salinity dilutions and its free amino acids at each dilution analyzed. In this case, each species represents a particular portion of the salinity range from fresh water to the open ocean. Thus, species I (Diaptomus) is the fresh water representative. Species II-IV are "estuarine and marine" copepods which prefer increasingly higher salinities for propagation. Of these, species II (Eurytemora affinis) is the representative of the low salinity environment which occurs in the upper Pettaquamscutt River (Tables 1 and 15, Appendix II). Species III (Acartia tonsa) and species IV (Acartia clausi) both occur in Narragansett Bay, yet the latter is more successful in the lower parts of the Bay where salinities are higher (Jeffries, 1962b). Species V (Pseudocalanus minutus) prefers the more saline waters near the mouth of the Bay and in Rhode Island Sound. Species VI (Calanus finmarchicus) is most abundant in the high salinity waters of the open ocean.

The distribution of these species along the salinity gradient is shown in Table 1. Although the salinity gradient from Narragansett Bay to Rhode Island Sound and the open ocean amounts to no more than 8 o/oo (25-33 o/oo), the gradient appears to control the overlapping yet distinct distribution of each species. In fact, moving from the open ocean into the Bay, one encounters a succession of species populations, each adapted to segments of the overall salinity range. Thus, the free amino acid concentrations of these six species were correlated with the portion of the salinity range in which each species propagates most successfully.

Figure 1 illustrates that as the environment becomes increasingly more saline there appears to be an increase in the mean concentrations of most free amino acids. E. affinis, the brackish water copepod of Pettaquamscutt River, has higher concentrations than Diaptomus, the fresh water species. The final estimate of error (Table 3) indicates, however, that the two species may have similar concentrations of aspartic acid, valine, threonine, tyrosine, and lysine. This is shown by the vertical limits ($\pm$ the final estimate of error) on the means in Figure 1. Likewise, the estuarine copepods of Narragansett Bay, A. tonsa and A. clausi, have higher concentrations of free amino acids than E. affinis, but this latter species and A. tonsa may be similar in their concentrations of methionine, cysteic acid, glutamic acid, tyrosine, phenylalanine and alanine. The free amino acid concentrations of A. clausi and P. minutus appear markedly similar as shown by the high incidence of overlapping vertical ranges (Figure 1). The two species may differ only in their concentrations of cysteic acid, aspartic acid, glutamic acid, lysine, alanine and arginine. With the exception of the free amino acid values of P. minutus which are consistently lower or overlap with those of A. clausi, the increase in the concentration of most free amino acids with the increasing salinity of the environment appears to be a linear function.

However, going from a fresh water species to a marine species, one finds that the greatest increases occur in the concentrations of the most abundant free amino acids: taurine, proline, glycine, glutamic acid, alanine, and arginine (Figure 1). These particular amino acids appear to increase in a curvilinear manner with the increasing salinity of the environment. Maximum taurine, proline, and glycine concentrations

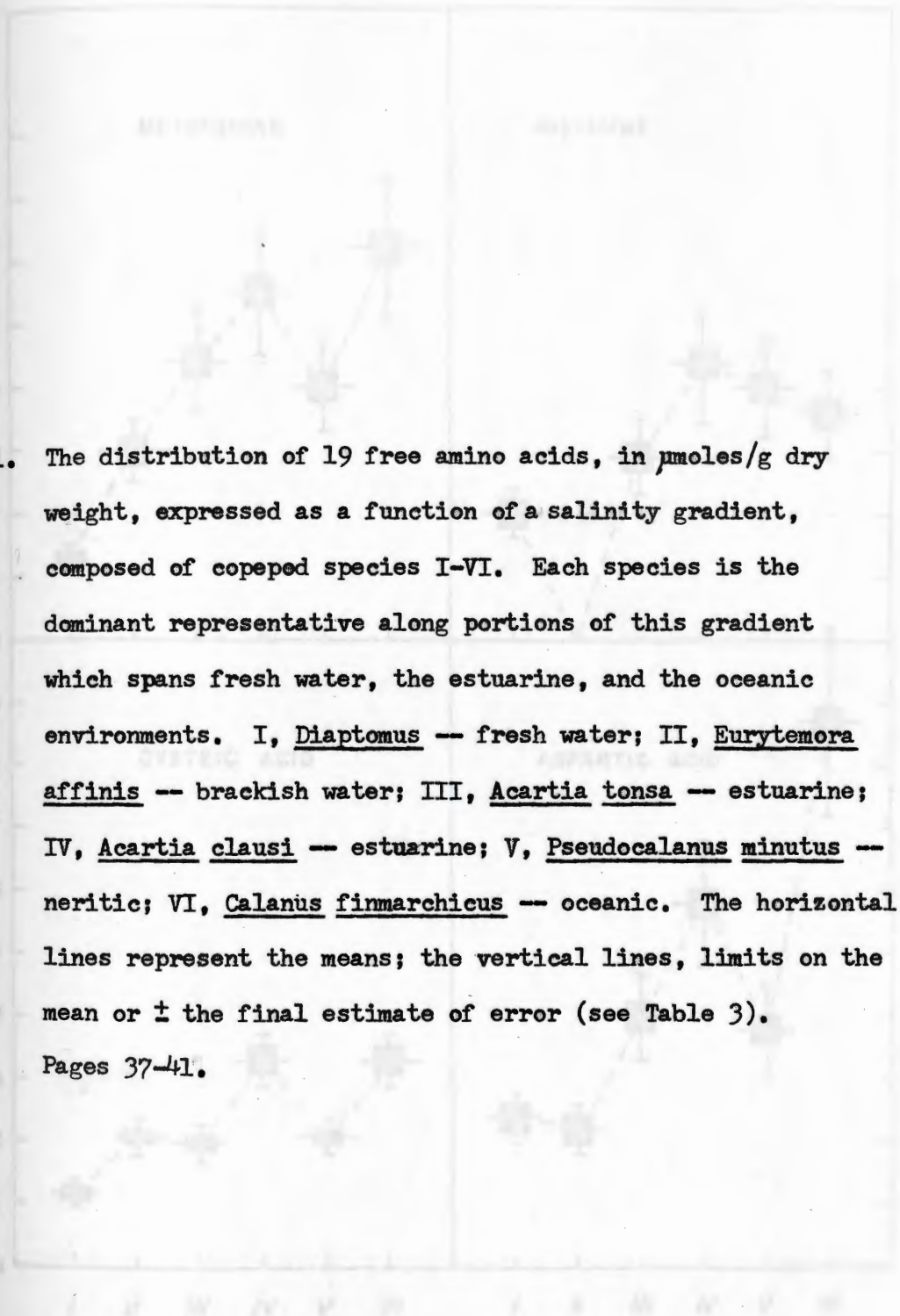

Figure 1. The distribution of 19 free amino acids, in μmoles/g dry weight, expressed as a function of a salinity gradient, composed of copeped species I-VI. Each species is the dominant representative along portions of this gradient which spans fresh water, the estuarine, and the oceanic environments. I, Diaptomus -- fresh water; II, Eurytemora affinis -- brackish water; III, Acartia tonsa -- estuarine; IV, Acartia clausi -- estuarine; V, Pseudocalanus minutus -- neritic; VI, Calanus finmarchicus -- oceanic. The horizontal lines represent the means; the vertical lines, limits on the mean or ± the final estimate of error (see Table 3).

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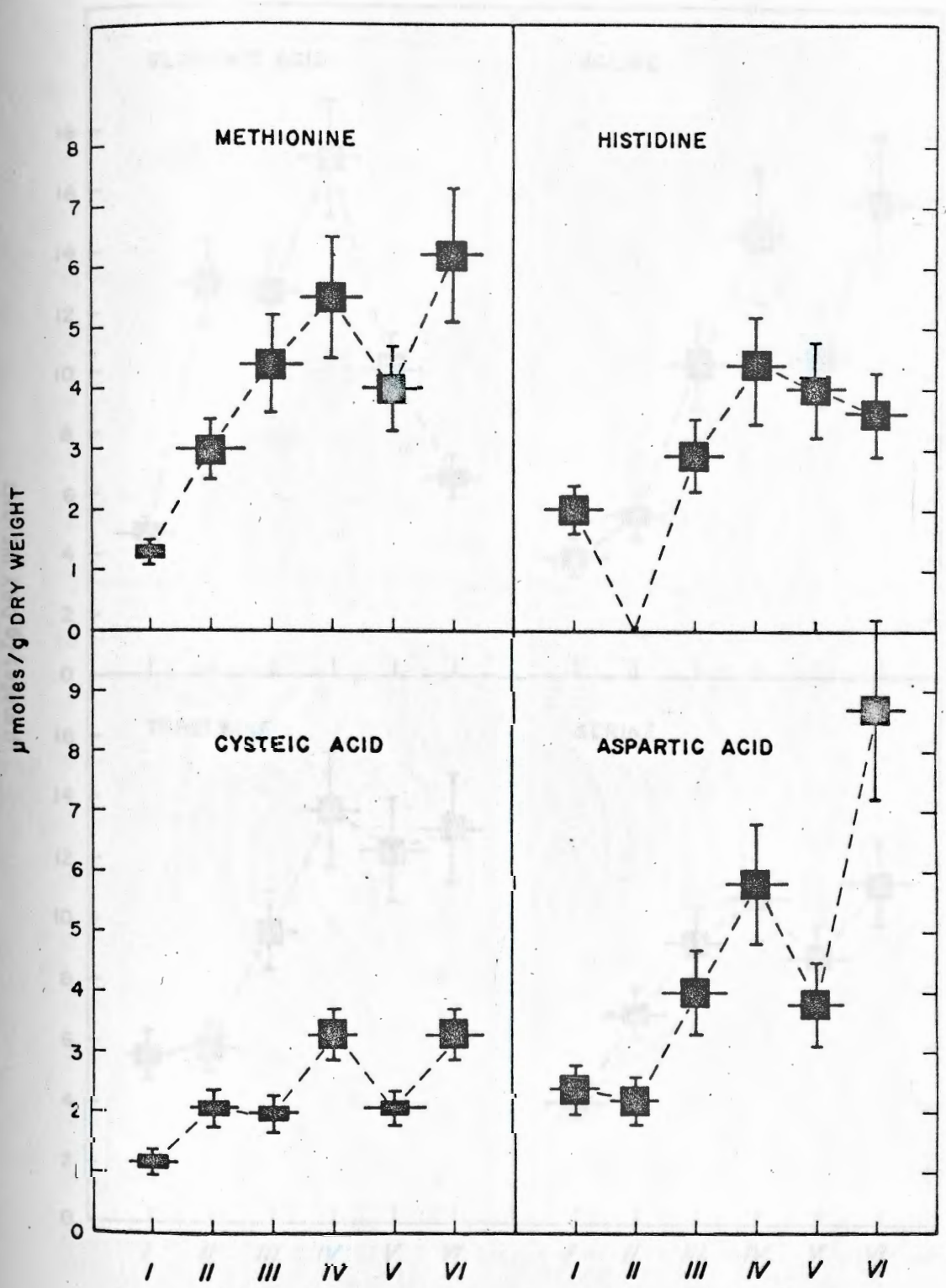
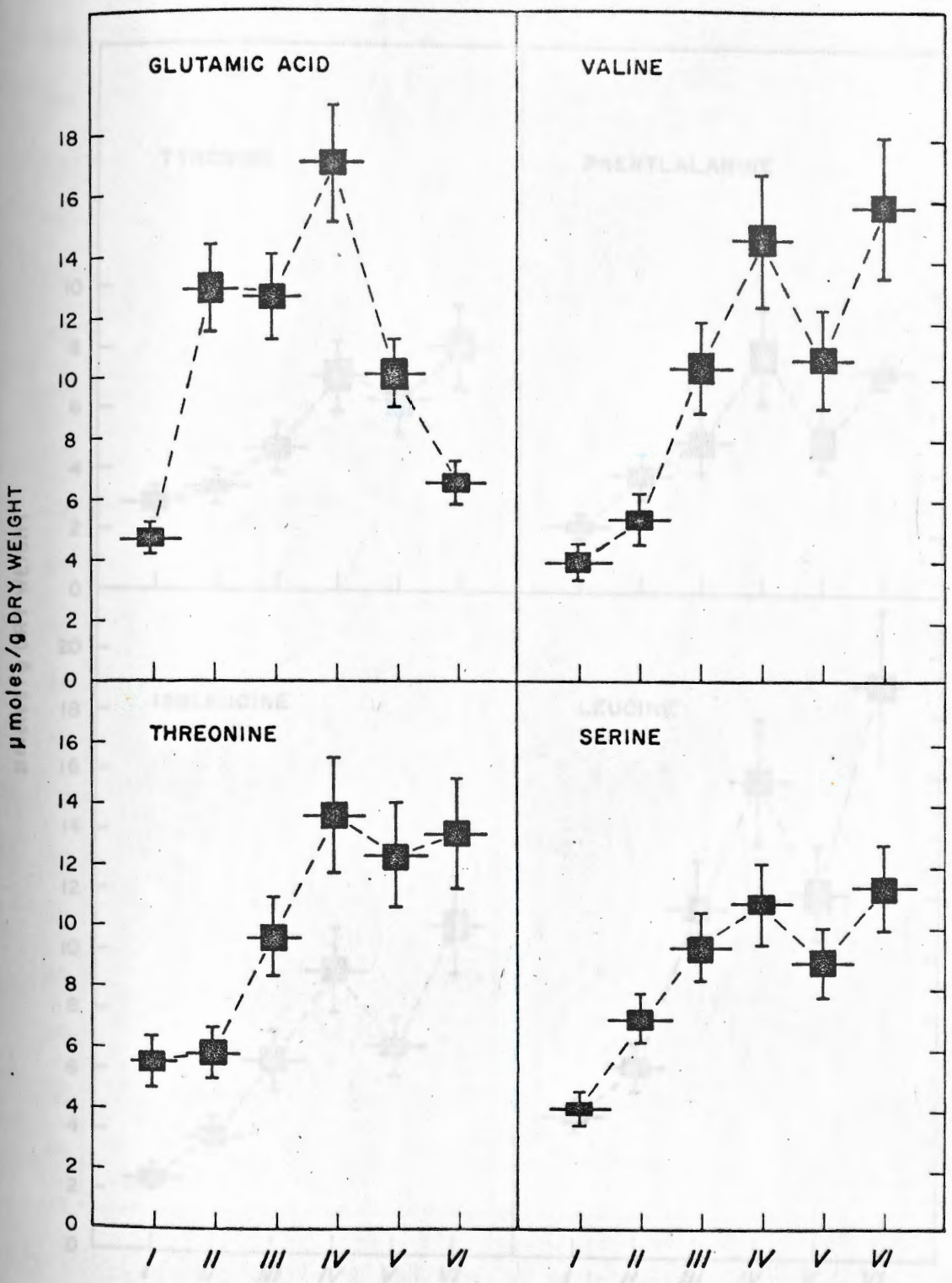
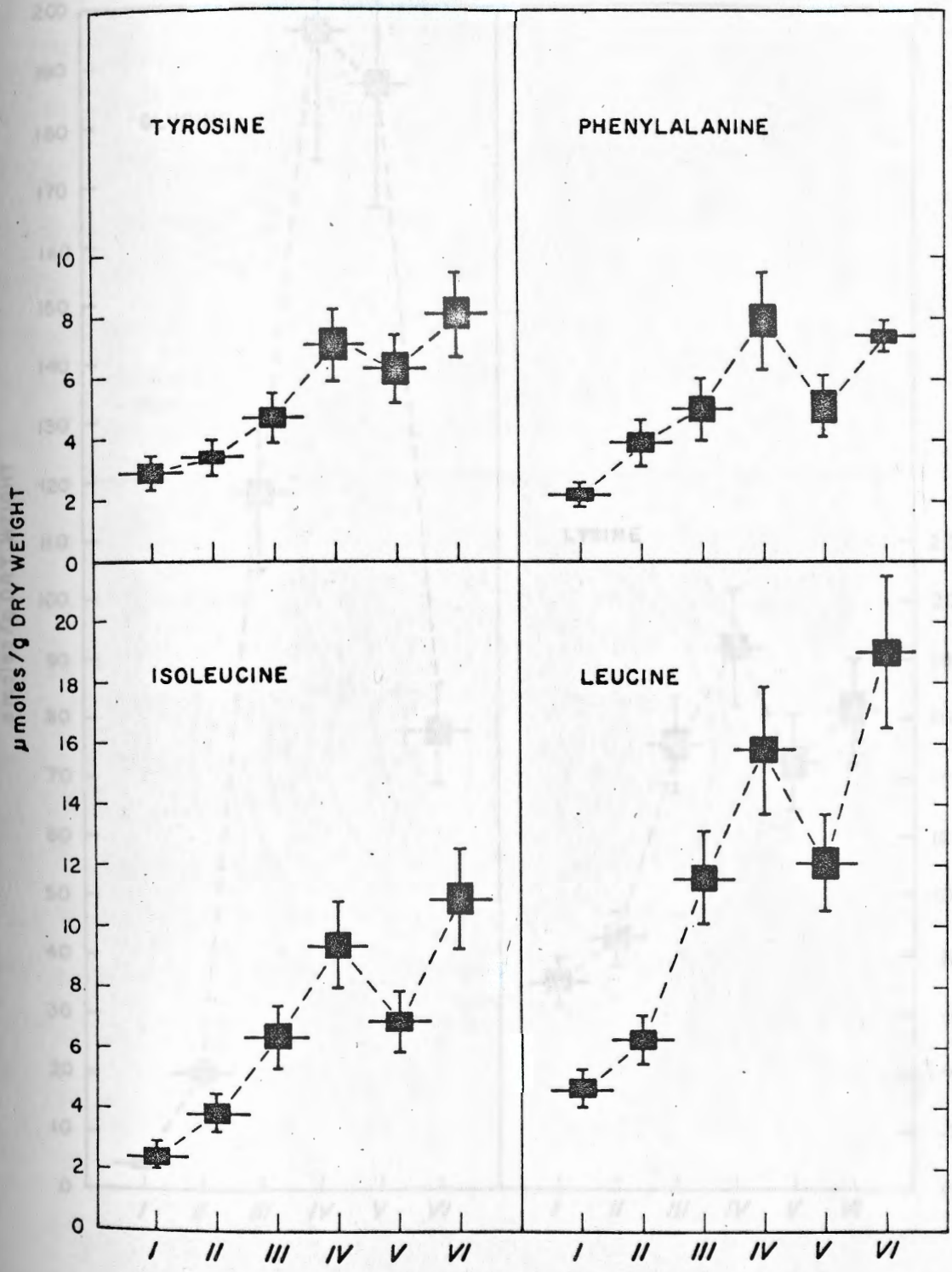
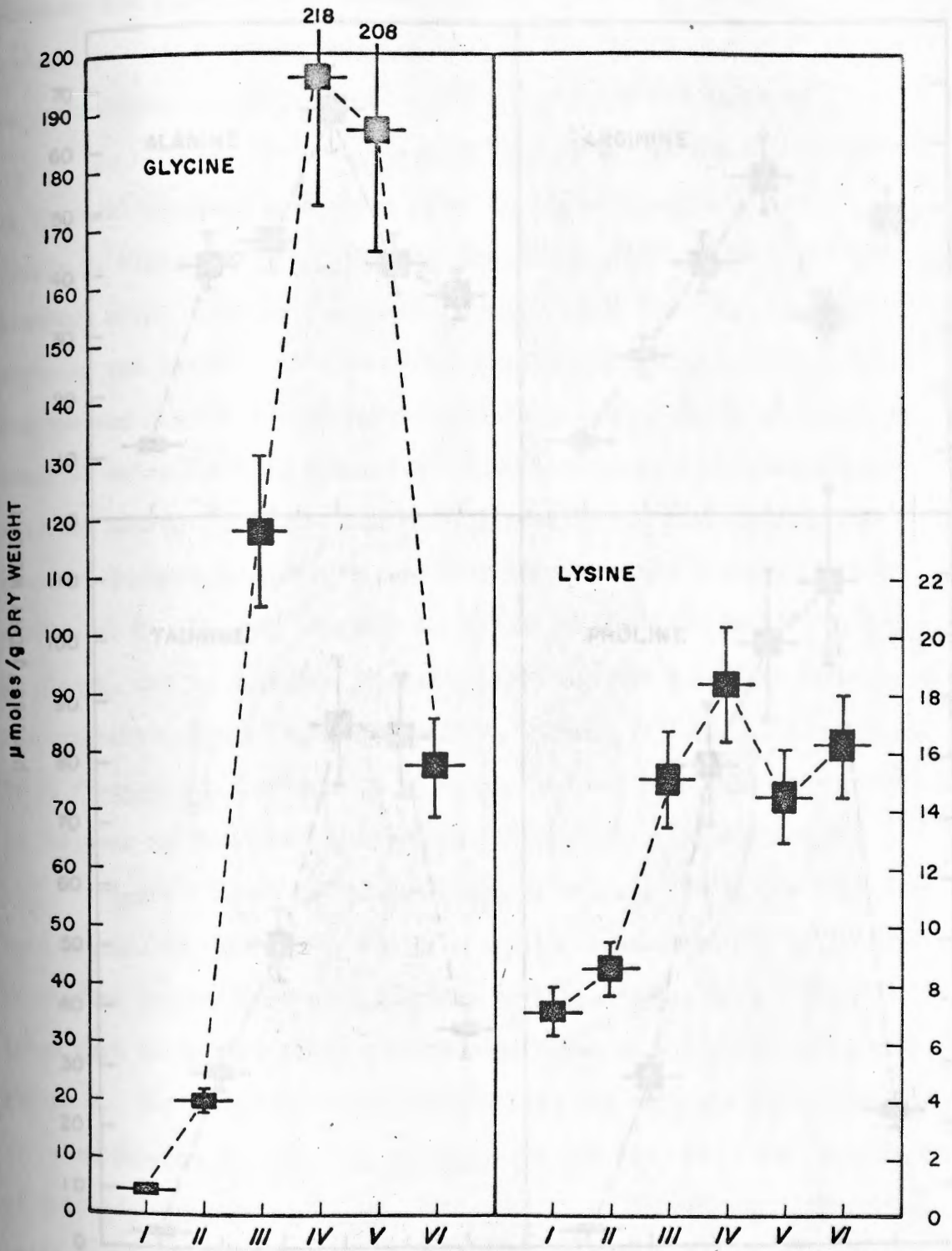
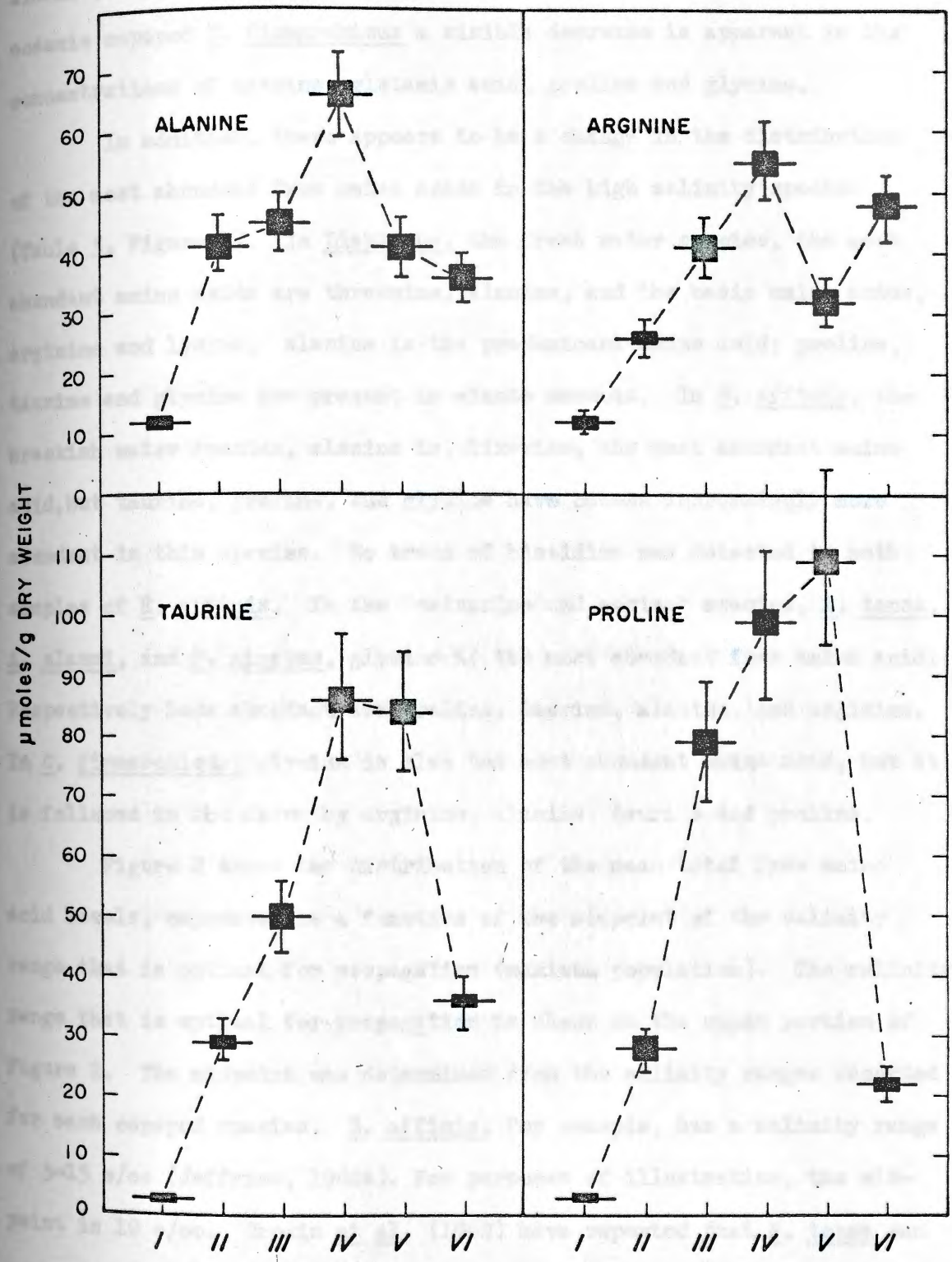

METHIONINE
HISTIDINE
CYSTEIC ACID
ASPARTIC ACID
μ moles/g DRY WEIGHT
0
1
2
3
4
5
6
7
8
9
I
II
III
IV
V
VI


GLUTAMIC ACID
VALINE
THREONINE
SERINE
μ moles/g DRY WEIGHT
0
2
4
6
8
10
12
14
16
18
I
II
III
IV
V
VI


TYROSINE
PHENYLALANINE
ISOLEUCINE
LEUCINE
µmoles/g DRY WEIGHT
0
2
4
6
8
10
12
14
16
18
20
I
II
III
IV
V
VI


218
208
GLYCINE
LYSINE
µ moles/g DRY WEIGHT
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
22
20
18
16
14
12
10
8
6
4
2
0
I
II
III
IV
V
VI


ALANINE
ARGININE
TAURINE
PROLINE
µmoles/g DRY WEIGHT
0
10
20
30
40
50
60
70
80
90
100
110
I
II
III
IV
V
VI

are found in A. clausi and P. minutus. Maximum arginine, lysine, alanine, and glutamic acid concentrations occur in A. clausi. In the oceanic copepod C. finmarchicus a visible decrease is apparent in the concentrations of taurine, glutamic acid, proline and glycine.

In addition, there appears to be a change in the distribution of the most abundant free amino acids in the high salinity species (Table 5, Figure 1). In Diaptomus, the fresh water species, the most abundant amino acids are threonine, alanine, and the basic amino acids, arginine and lysine. Alanine is the predominant amino acid; proline, taurine and glycine are present in minute amounts. In E. affinis, the brackish water species, alanine is, likewise, the most abundant amino acid, but taurine, proline, and glycine have become increasingly more abundant in this species. No trace of histidine was detected in both samples of E. affinis. In the "estuarine and marine" species, A. tonsa, A. clausi, and P. minutus, glycine is the most abundant free amino acid. Respectively less abundant are proline, taurine, alanine, and arginine. In C. finmarchicus, glycine is also the most abundant amino acid, but it is followed in abundance by arginine, alanine, taurine and proline.

Figure 2 shows the distribution of the mean total free amino acid levels, expressed as a function of the midpoint of the salinity range that is optimal for propagation (maximum population). The salinity range that is optimal for propagation is shown on the upper portion of Figure 2. The midpoint was determined from the salinity ranges reported for each copepod species. E. affinis, for example, has a salinity range of 5-15 o/oo (Jeffries, 1962a). For purposes of illustration, the midpoint is 10 o/oo. Cronin et al. (1962) have reported that A. tonsa can produce large endemic populations in a salinity range of 5-30 o/oo. How-

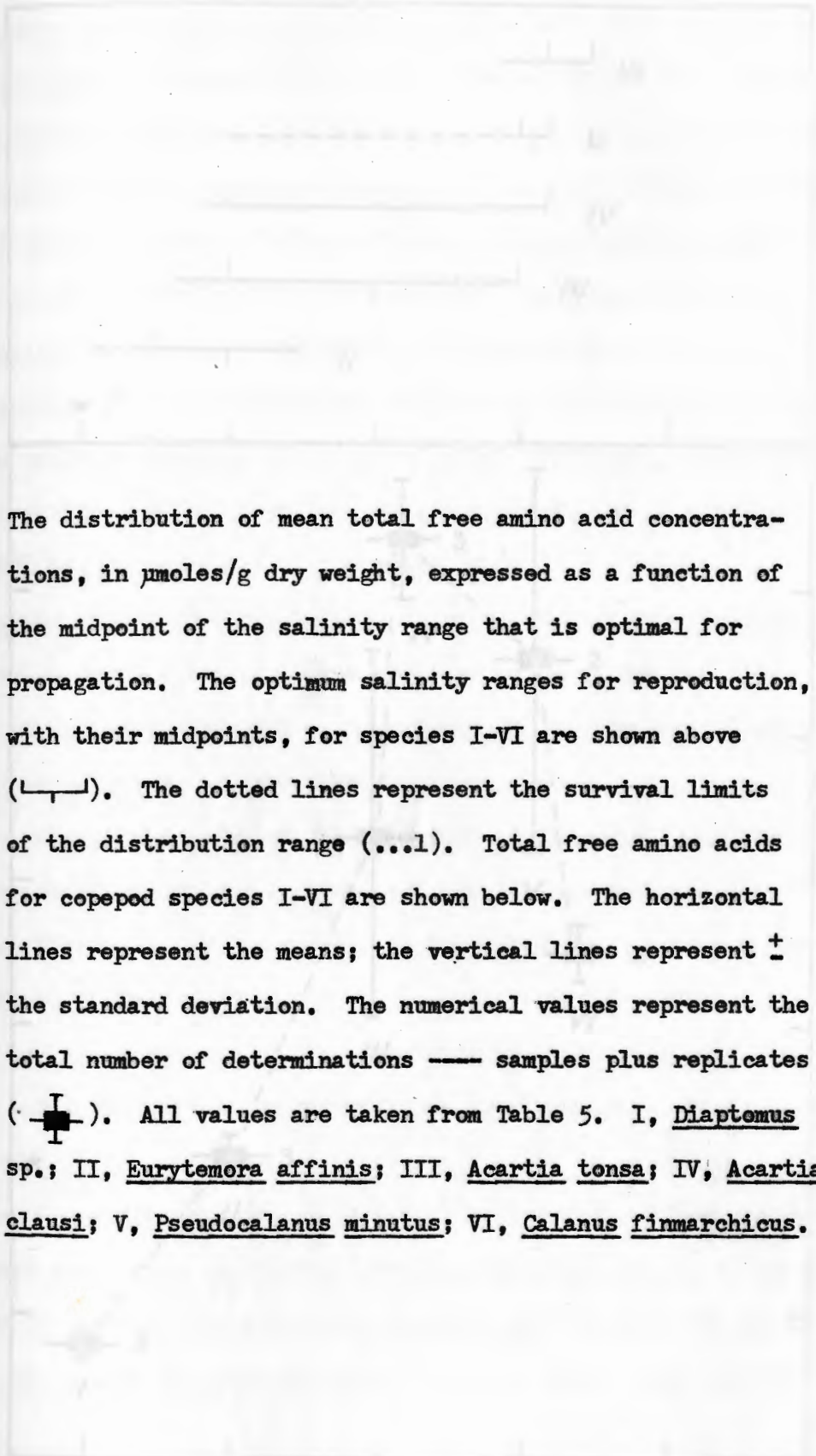
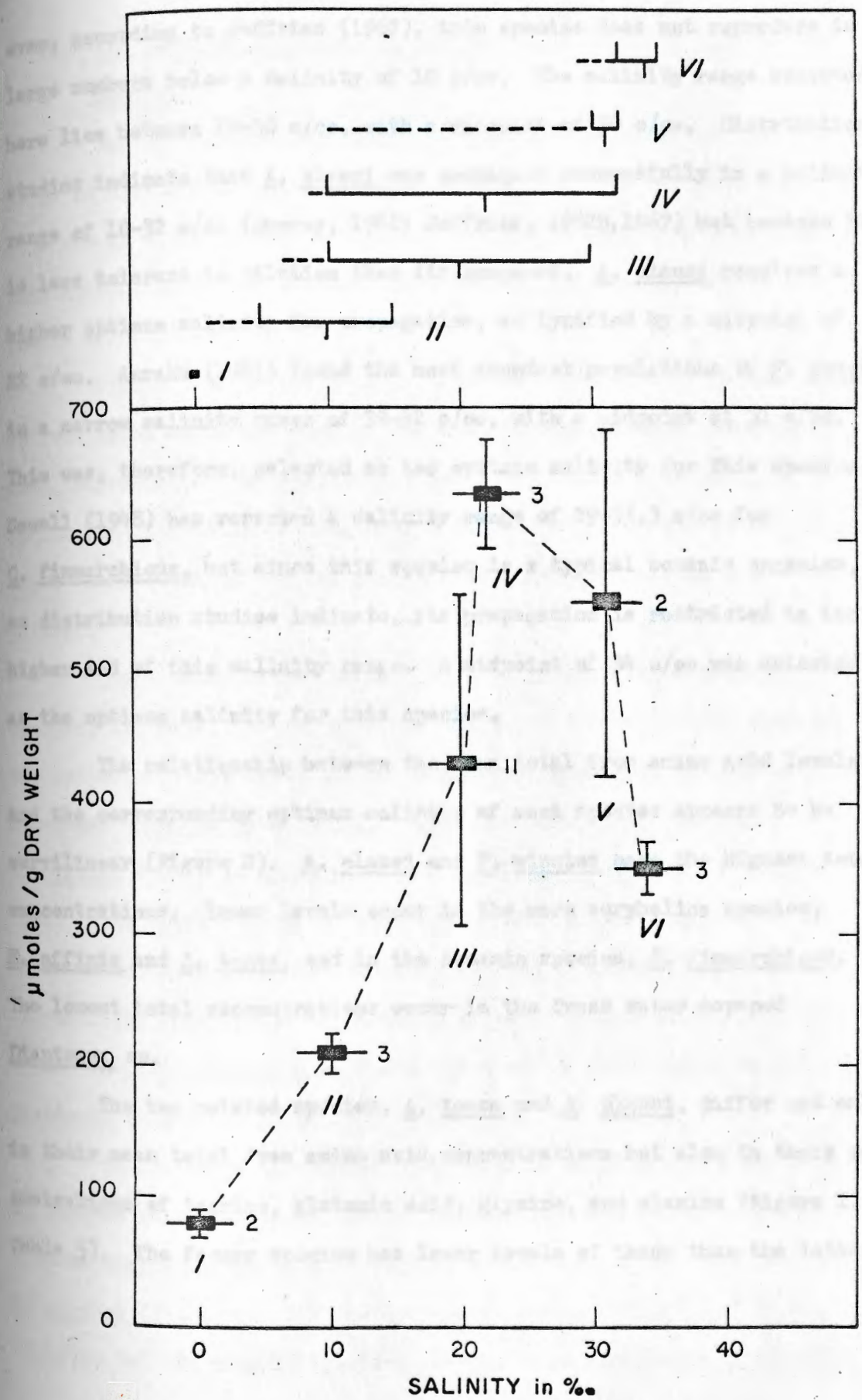


Figure 2. The distribution of mean total free amino acid concentrations, in μmoles/g dry weight, expressed as a function of the midpoint of the salinity range that is optimal for propagation. The optimum salinity ranges for reproduction, with their midpoints, for species I-VI are shown above (└┬┘). The dotted lines represent the survival limits of the distribution range (...). Total free amino acids for copepod species I-VI are shown below. The horizontal lines represent the means; the vertical lines represent ± the standard deviation. The numerical values represent the total number of determinations ---- samples plus replicates (·┼·). All values are taken from Table 5. I, Diaptomus sp.; II, Eurytemora affinis; III, Acartia tonsa; IV, Acartia clausi; V, Pseudocalanus minutus; VI, Calanus finmarchicus.


VII
V
IV
III
II
I
700
600
500
400
300
200
100
0
3
2
IV
II
V
3
VI
III
3
II
2
I
0
10
20
30
40
µ moles / g DRY WEIGHT
SALINITY in ‰

ever, according to Jeffries (1967), this species does not reproduce in large numbers below a salinity of 10 o/oo. The salinity range selected here lies between 10-30 o/oo, with a midpoint of 20 o/oo. Distribution studies indicate that A. clausi can propagate successfully in a salinity range of 10-32 o/oo (Deevey, 1948; Jeffries, 1962b,1967) but because it is less tolerant to dilution than its congener, A. clausi requires a higher optimum salinity for propagation, as typified by a midpoint of 22 o/oo. Anraku (1961) found the most abundant populations of P. minutus in a narrow salinity range of 30-32 o/oo, with a midpoint at 31 o/oo. This was, therefore, selected as the optimum salinity for this species. Sewell (1948) has reported a salinity range of 29-35.3 o/oo for C. finmarchicus, but since this species is a typical oceanic organism, as distribution studies indicate, its propagation is restricted to the higher end of this salinity range. A midpoint of 34 o/oo was selected as the optimum salinity for this species.

The relationship between the mean total free amino acid levels and the corresponding optimum salinity of each species appears to be curvilinear (Figure 2). A. clausi and P. minutus have the highest total concentrations. Lower levels occur in the more euryhaline species, E. affinis and A. tonsa, and in the oceanic species, C. finmarchicus. The lowest total concentrations occur in the fresh water copepod Diaptomus sp.

The two related species, A. tonsa and A. clausi, differ not only in their mean total free amino acid concentrations but also in their concentrations of taurine, glutamic acid, glycine, and alanine (Figure 1, Table 5). The former species has lower levels of these than the latter.

4. The Relative Distribution of Free Amino Acids in Six Copepod Species

The relative distribution, in mean per cents, of 19 free amino acids is listed for the six copepod species in Table 6. Each value was obtained by dividing the mean amino acid concentration by the total amino acid concentration and multiplying by 100. The data of Table 5 were used in the calculations.

Figure 3 shows the overall, relative distribution pattern of the 19 free amino acids for each of the six copepod species. Each amino acid, expressed as per cent of the total free amino acid pool, is plotted on its own axis which extends from 0 at the origin to 30% at the outer edge. Subsequently, all the points were connected to form the observed pattern.

Eight amino acids, consisting of glycine, proline, taurine, alanine, arginine, lysine, glutamic acid, and aspartic acid, comprise the following proportions of the total free amino acid pool in the six copepods: *Diaptomus*, 61.3%; *E. affinis*, 80.1%; *A. tonsa*, 84.5%; *A. clausi*, 85.6%; *P. minutus*, 87.0%; *C. finmarchicus*, 71.8% (Figure 4). Thus, there appears to be an increase in the relative proportion of these amino acids as the environment changes from fresh water to the increasingly more saline environment of Rhode Island Sound. The relative values in *C. finmarchicus* approach those of the fresh water species *Diaptomus* (Figure 4).

The relative distribution patterns of glycine and alanine bear an inverse relationship in the six copepod species (Table 6, Figure 4). Glycine comprises the largest portion of the free amino acid pool in *P. minutus* (33.8%) and the smallest in *E. affinis* (9.5%) and *Diaptomus* (5.7%). But the highest relative alanine values occur in *E. affinis*

Table 6. A comparison of the free amino acid composition of copepod species, expressed as per cent of total free amino acid pool, using data of Table 5. Values represent mean per cents (%). N is the number of samples analyzed.

AMINO ACID	Diaptomus %	Eurytemora affinis %	Acartia tonsa %	Acartia clausi %	Pseudocalanus minutus %	Calanus finmarchicus %
CYS	1.5	1.0	0.5	0.5	0.4	0.9
TAU	3.7	14.0	11.5	13.6	15.1	10.2
ASP	3.0	1.1	0.9	0.9	0.7	2.5
THR	6.7	2.8	2.2	2.1	2.2	3.7
SER	5.0	3.3	2.2	1.7	1.6	3.2
GLU	5.8	6.1	2.9	2.7	1.8	1.9
PRO	4.0	13.2	18.3	15.5	19.8	6.4
GLY	5.7	9.5	27.4	30.9	33.8	22.2
ALA	15.4	19.6	10.5	10.4	7.4	10.3
VAL	4.8	2.5	2.4	2.3	1.9	4.5
METH	1.6	1.4	1.0	0.9	0.7	1.8
ILEU	3.1	1.9	1.5	1.5	1.2	3.1
LEU	5.8	3.0	2.7	2.5	2.2	5.4
TYR	3.6	1.6	1.1	1.1	1.1	2.3
PHE	2.7	1.9	1.2	1.2	0.9	2.1
ORN	0.6	0.6	+	+	+	0.3
LYS	8.8	4.1	3.5	2.9	2.6	4.7
HIS	2.5	-----	0.7	0.7	0.7	1.0
ARG	14.9	12.5	9.5	8.7	5.8	13.6
N	1	2	6	3	2	1

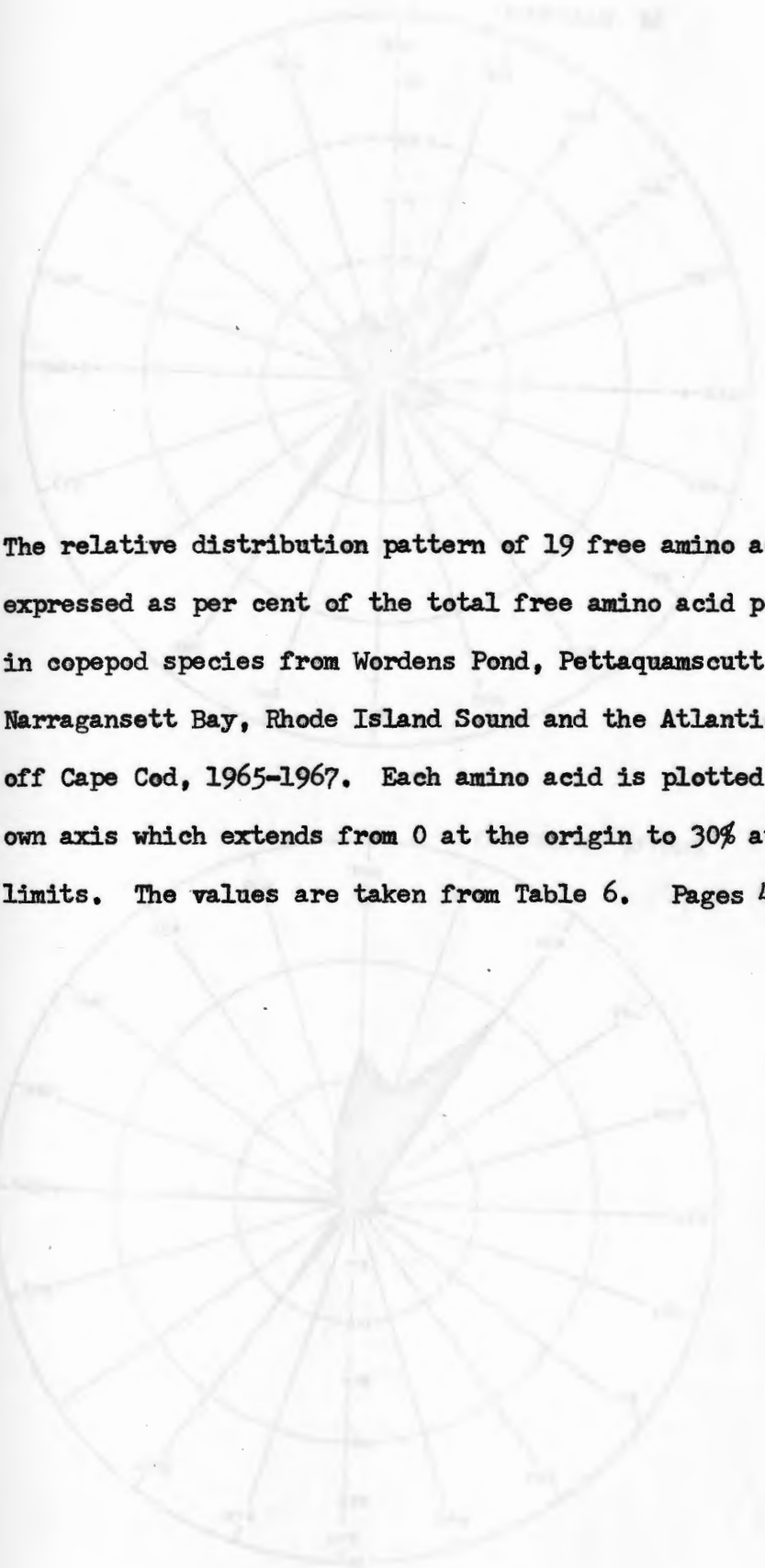
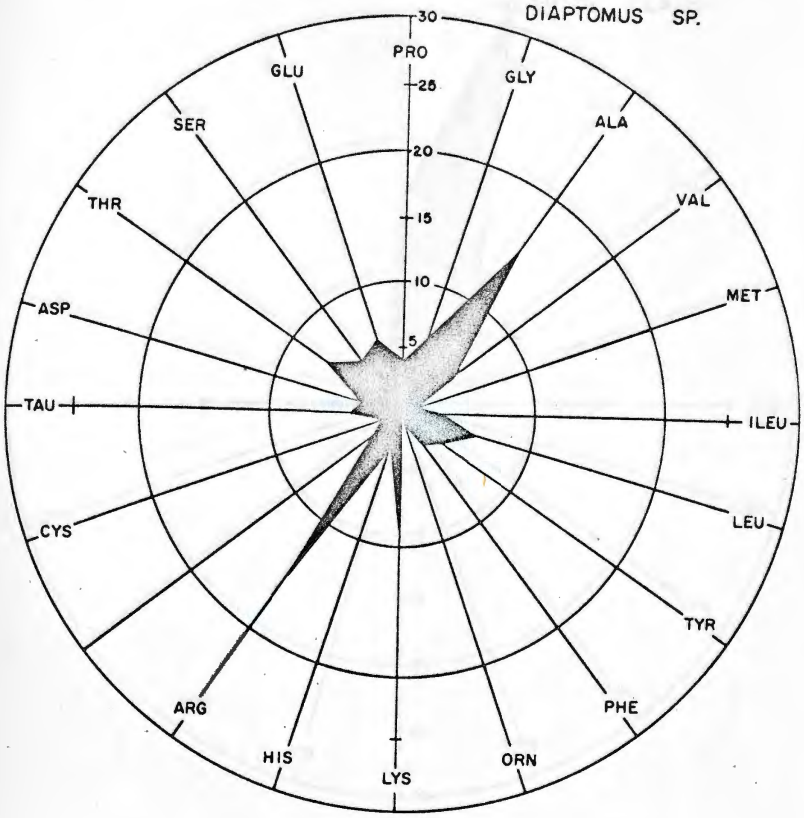
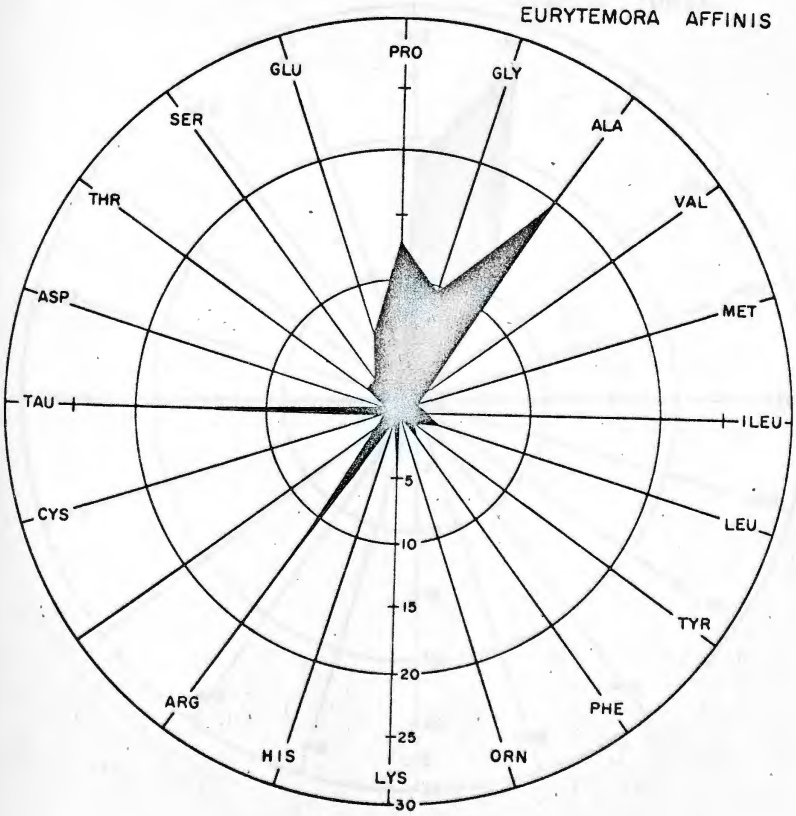
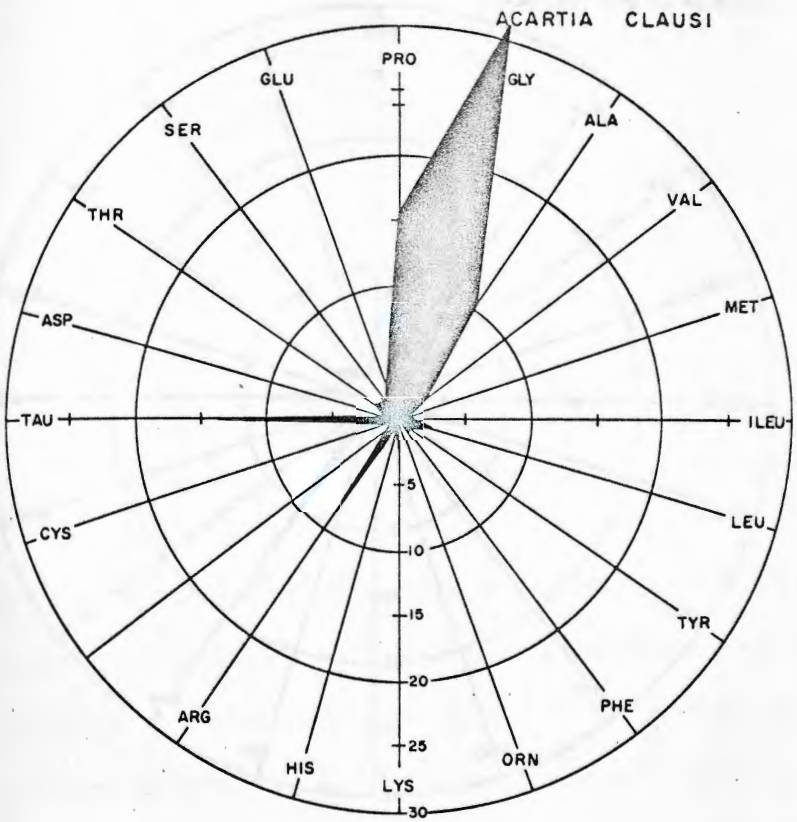
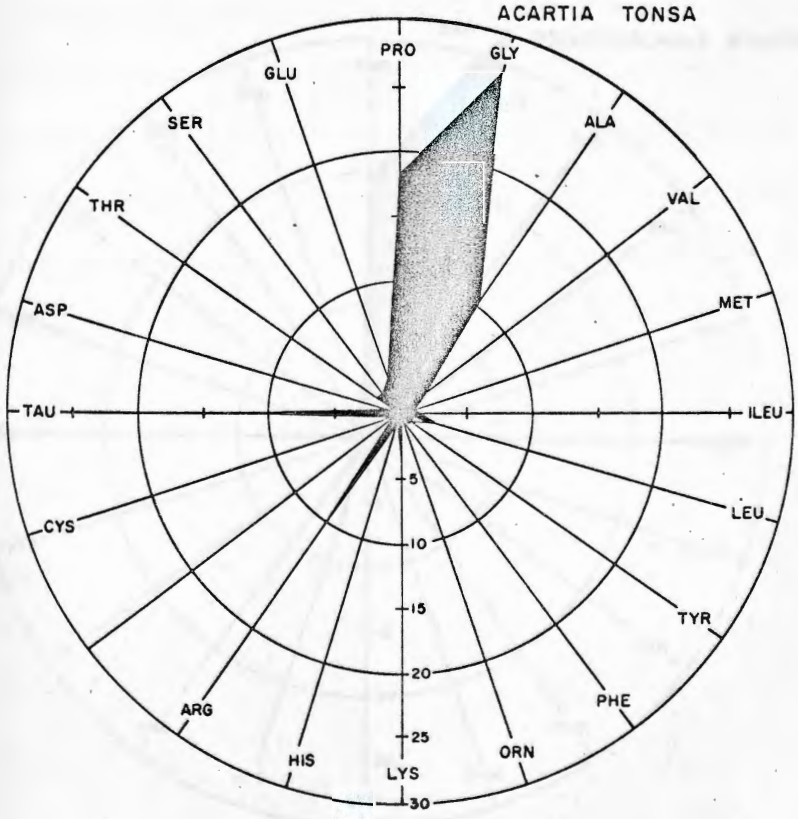


Figure 3. The relative distribution pattern of 19 free amino acids, expressed as per cent of the total free amino acid pool, in copepod species from Wordens Pond, Pettaquamscutt River, Narragansett Bay, Rhode Island Sound and the Atlantic Ocean, off Cape Cod, 1965-1967. Each amino acid is plotted on its own axis which extends from 0 at the origin to 30% at its limits. The values are taken from Table 6. Pages 49-51.

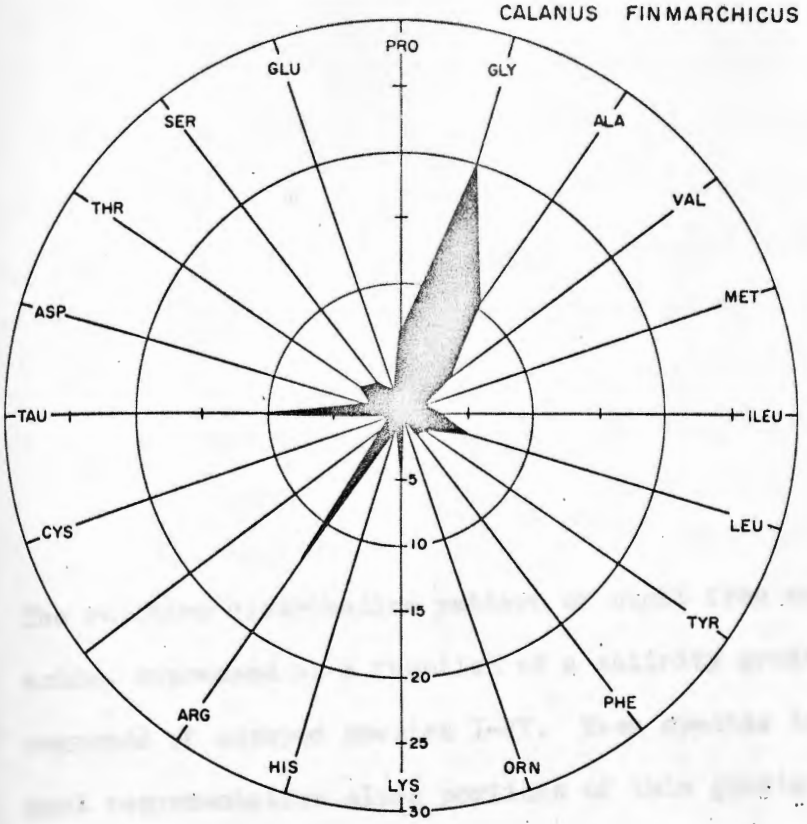

DIAPTOMUS SP.
30
PRO
25
GLY
ALA
20
VAL
15
MET
10
ILEU
5
LEU
TYR
PHE
ORN
LYS
HIS
ARG
CYS
TAU
ASP
THR
SER
GLU


EURYTEMORA AFFINIS
PRO
GLY
ALA
VAL
MET
ILEU
LEU
TYR
PHE
ORN
LYS
HIS
ARG
CYS
TAU
ASP
THR
SER
GLU
5
10
15
20
25
30

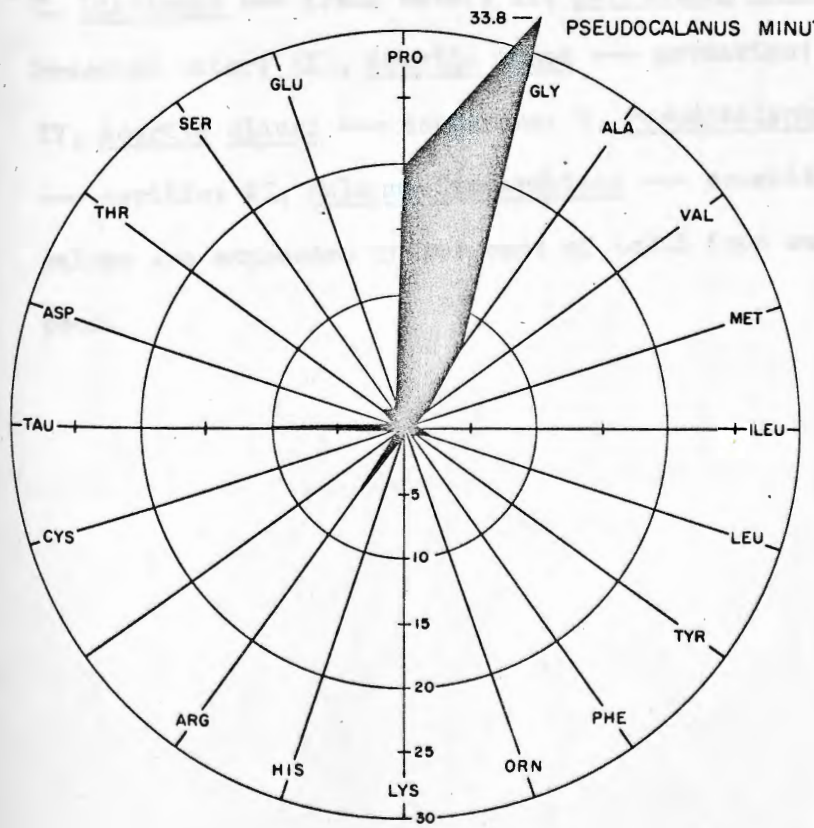

ACARTIA CLAUSI
PRO
GLY
ALA
VAL
MET
ILEU
LEU
TYR
PHE
ORN
LYS
HIS
ARG
CYS
TAU
ASP
THR
SER
GLU
5
10
15
20
25
30


ACARTIA TONSA
PRO
GLY
ALA
VAL
MET
ILEU
LEU
TYR
PHE
ORN
LYS
HIS
ARG
CYS
TAU
ASP
THR
SER
GLU
5
10
15
20
25
30

CALANUS FINMARCHICUS



PSEUDOCALANUS MINUTUS



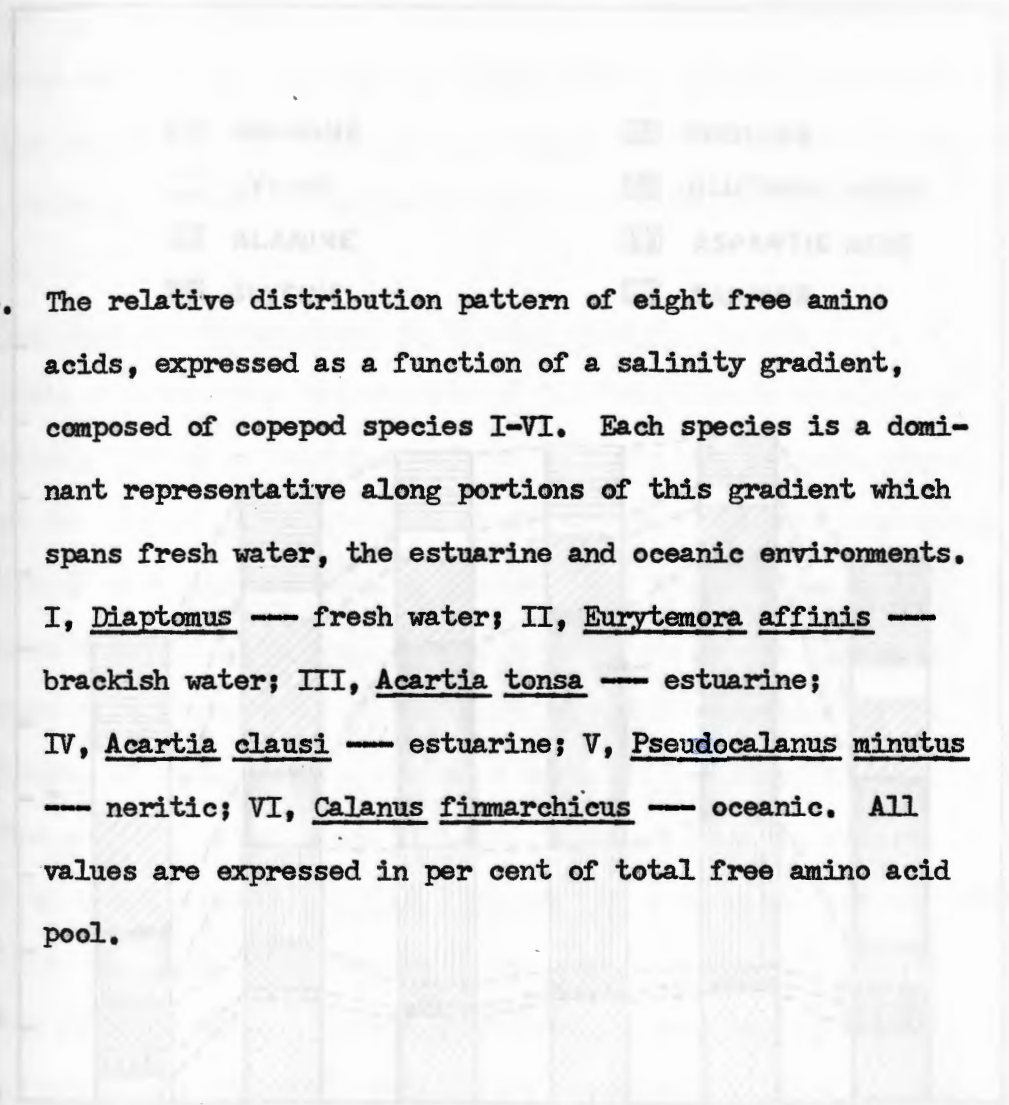
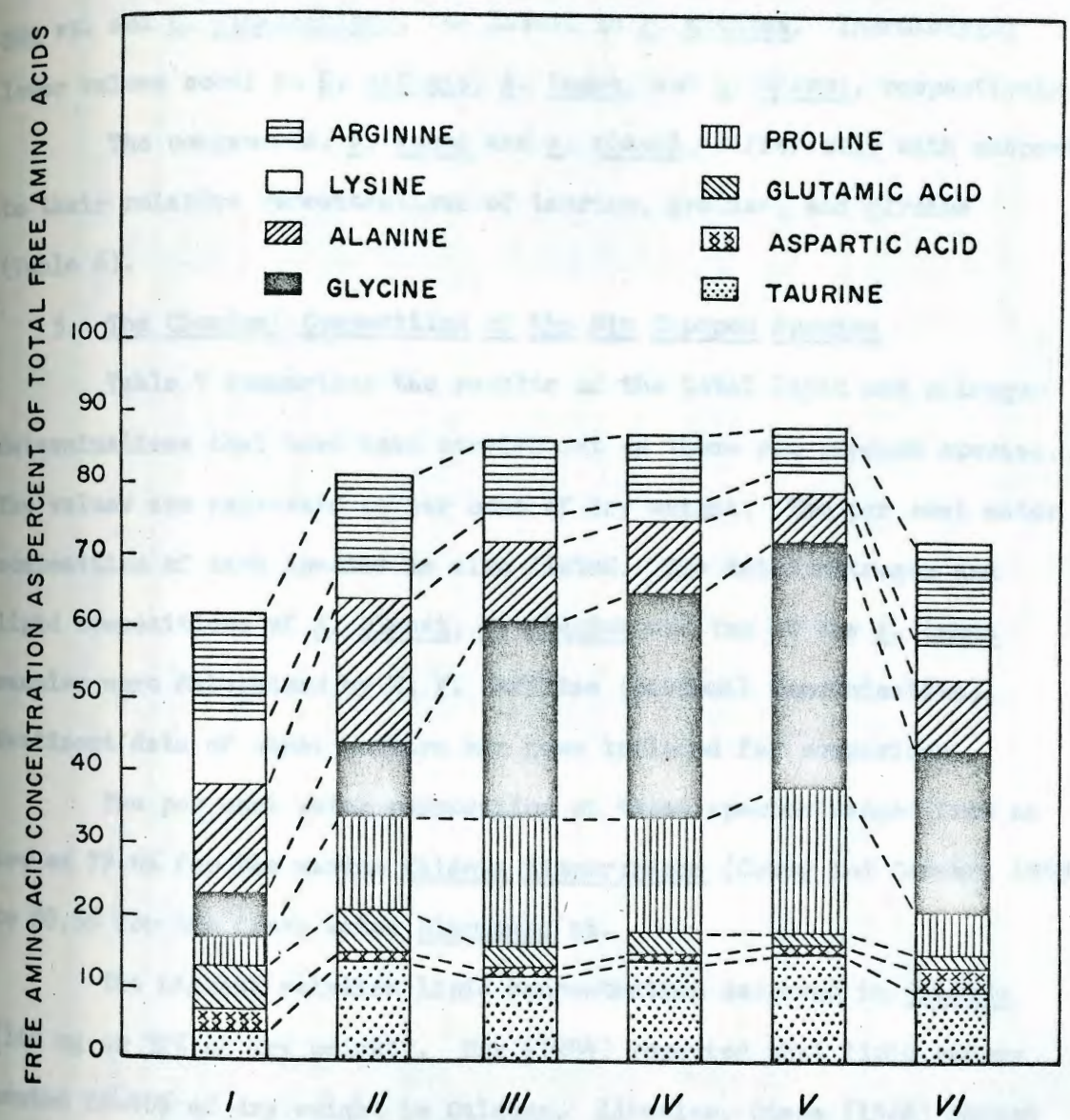


Figure 4. The relative distribution pattern of eight free amino acids, expressed as a function of a salinity gradient, composed of copepod species I-VI. Each species is a dominant representative along portions of this gradient which spans fresh water, the estuarine and oceanic environments. I, Diaptomus --- fresh water; II, Eurytemora affinis --- brackish water; III, Acartia tonsa --- estuarine; IV, Acartia clausi --- estuarine; V, Pseudocalanus minutus --- neritic; VI, Calanus finmarchicus --- oceanic. All values are expressed in per cent of total free amino acid pool.


ARGININE
LYSINE
ALANINE
GLYCINE
PROLINE
GLUTAMIC ACID
ASPARTIC ACID
TAURINE
FREE AMINO ACID CONCENTRATION AS PERCENT OF TOTAL FREE AMINO ACIDS
100
90
80
70
60
50
40
30
20
10
0
I
II
III
IV
V
VI

(19.6%) and Diaptomus (15.4%) and the lowest in P. minutus (7.4%). The relative distribution of glutamic acid is similar to that of alanine.

The relative distributions of isoleucine, leucine, valine, serine, threonine, aspartic acid, arginine, lysine, methionine, and cysteic acid are similar (Table 6). The highest values occur in Diaptomus sp. and C. finmarchicus, the lowest in P. minutus. Increasingly lower values occur in E. affinis, A. tonsa, and A. clausi, respectively.

The congeneres, A. tonsa and A. clausi, differ only with respect to their relative concentrations of taurine, proline, and glycine (Table 6).

5. The Chemical Composition of the Six Copepod Species

Table 7 summarizes the results of the total lipid and nitrogen determinations that have been carried out on these six copepod species. The values are expressed as per cent of dry weight. The per cent water composition of each species is also listed. The total nitrogen and lipid compositions of A. clausi, P. minutus and two of the A. tonsa samples were determined by H. P. Jeffries (personal communication). Pertinent data of other workers has been included for comparison.

The per cent water composition of these species ranged from as low as 77.6% for the marine Calanus finmarchicus (Cowey and Corner, 1963) to 88.8% for the fresh water Diaptomus sp.

The highest relative lipid concentration occurred in Calanus (166 mg or 32% of dry weight). Orr (1934) reported that lipid represented 20-40% of dry weight in Calanus. Likewise, Giese (1966) showed that the lipid fraction of Calanus from the north represented 20-28% of dry weight. There is a relatively close agreement between the lipid values of other workers and those reported for Calanus in this study.

Table 7. *The chemical composition of copepod species from Wordens Pond, Watchaug Pond, Pettaquamscutt River, Narragansett Bay, Rhode Island Sound and the Atlantic Ocean (10 miles north of Provincetown, Massachusetts). The total nitrogen and lipid concentrations are expressed in milligrams (mg) and per cent (%) of dry weight. October, 1965 - August, 1967.*

Species	Date	Location	Total Nitrogen mg	% Nitrogen	Total Lipid mg	% Lipid	% Water
Diaptomus	Aug. 9	Wordens Pond	-----	-----	77.0	15.39	84.57
	June 14	Watchaug Pond	-----	-----	48.0	15.93	88.80
	May 1-8	Lake Mendota[a]	-----	9.27-9.87	----	16-40	-----
Eurytemora affinis	Feb. 9	Pett. R.	-----	-----	88.4	17.33	86.30
	Mar. 8		-----	-----	94.1	17.62	-----
Acartia tonsa	Dec. 6*	Nar. B.	2.80	8.62	59.6	11.92	83.70
	Aug. 17		-----	-----	51.1	9.72	-----
	Oct. 18		2.80	10.40	-----	-----	-----
Acartia	Dec. 1*	Nar. B.	3.05	10.30	74.0	14.96	88.54
	Dec. 29*		3.62	10.80	71.7	14.26	83.85
	Apr. 20*		4.40	10.58	92.4	17.30	86.60
	Feb. 28	Nar. B.	-----	-----	78.5	14.74	-----
	Apr. 18	Pett. R.	-----	-----	91.8	18.29	87.88
Pseudocalanus minutus	Dec. 29*	RIS	3.12	10.40	65.8	13.10	86.10
	Jan. 26*		4.04	10.25	91.5	17.72	88.50
Calanus finmarchicus	Aug. 17	Atlantic Ocean	-----	-----	165.86	31.89	-----
	May	Firth of Clyde[b]	3.96	16.90	-----	-----	77.60
		Barents Sea[c]	-----	8.86-10.93	-----	-----	-----
		England[d]	-----	8.0	-----	20-40[e]	
						22-28[f]	78.00[g]

*Data provided by H. P. Jeffries
[a]Birge and Juday (1922)
[b]Cowey and Corner (1963)
[c]Yudonova (1940)
[d]Marchall and Orr (1955)
[e]Orr (1934)
[f]Giese (1966)
[g]Marshall, Nicholls and Orr (1934)

With the exception of A. tonsa which had the lowest relative lipid concentration (10-12% of dry weight), the relative lipid values of the other four species were markedly similar. In the fresh water Diaptomus sp., lipid represented 15-16% of dry weight; in E. affinis, 17-18%; in A. clausi, 14-18% and in P. minutus, 13-18%.

Total micro-Kjeldahl nitrogen determinations were done on only a few of the samples. Similar relative nitrogen concentrations were found in A. tonsa (9-10% of dry weight) and A. clausi and P. minutus (10-11%). Cowey and Corner (1963) reported a total nitrogen value of 16.9% for C. finmarchicus. According to Yudonova (1940), the total nitrogen content of Calanus represented only 9-11% of dry weight. Likewise, Marshall and Orr (1955) reported a lower total nitrogen fraction for Calanus (8% of dry weight). Total nitrogen in the fresh water Diaptomus sp. was shown to represent 9-10% of the dry weight (Birge and Juday, 1922). The total nitrogen content of these six copepod species appears to be markedly similar.

IV. DISCUSSION

A. Absolute and Relative Free Amino Acid Distributions in the Six Copepod Species

Overall Composition. The six copepod species have markedly similar free amino acid compositions. With the exception of Eurytemora affinis, all contained the 19 amino acids which commonly occur in protein (Table 5, Figure 1). No free histidine was detected in the two E. affinis samples. Its absence may be related to the observation that a large percentage of the E. affinis samples were females, bearing egg sacs. But, on the basis of this study, it cannot be determined if the entire population of E. affinis is characterized by an absence of free histidine or if the females simply lack free histidine at certain stages in their life cycle. It may be mentioned that Cowey (1965) observed that the free amino acid pool of the muscles of the salmon, Salmo salar, lacked histidine during the spawning cycle.

Trace amounts of homoserine, tryptophan, and urea were also present in most of the samples. In addition, all six species contained six components which could not be readily identified. These occurred on the chromatographic record: (1) after cysteic acid; (2) before taurine; (3) after urea; (4) before aspartic acid; (5) before isoleucine; (6) and before ornithine.

Arginine. All species contained abundant amounts of arginine (Tables 5 and 6). This agrees well with the reports of other workers who have shown high concentrations of arginine to be characteristic of the muscle tissues of invertebrates (Kermack et al., 1955; Cowey, 1961). The presence of free arginine in the six copepod species may result from the hydrolysis of arginine phosphate, the invertebrate phosphagen

(Simpson et al., 1959). The arginine may be liberated from phosphoarginine during the extraction procedure (Bricteaux-Grégoire et al., 1964).

The highest relative arginine levels occur in the marine species Calanus finmarchicus (13.6% of the total free amino acid pool) and the fresh water Diaptomus sp. (14.9%). Kermack et al. (1955) found arginine to represent only 5.7% of the total alpha-amino nitrogen content of the lobster Homarus vulgaris. Their value is comparable to the relative arginine content of Pseudocalanus minutus (5.8%). Based on the data of Cowey and Corner (1963), a relative arginine value of 14.1% was computed for C. finmarchicus, which agrees well with the results reported in this study (Table 13, Appendix I).

In the fresh water copepod Diaptomus sp., glycine, alanine, arginine, proline, taurine, glutamic acid, aspartic acid, and lysine compose only 61.3% of the total free amino acid content. Alanine and arginine represent approximately one-third of this fraction, and if lysine is included three amino acids now compose more than one-half of the 61.3%. Thus, there is a greater concentration of basic amino acids in the fresh water copepod than in the estuarine and marine species (Figures 3 and 4). In fresh water, the major ions (sodium, potassium, chloride) are less concentrated and the ionic composition is more variable (Potts and Parry, 1964). As a result, a possible function of lysine and arginine may be to act as cations to balance the decreased potassium ion concentration in the cells. Based on his studies on potassium-deficient Fundulus heteroclitus, Hanlon (1960) suggested such a function for lysine.

Glycine. Both Acartia species, along with P. minutus and C. finmarchicus, are characterized by high concentrations of glycine,

taurine, proline, and alanine (Tables 5 and 6). Simpson et al. (1959) found high glycine and taurine concentrations in the muscle tissues of 17 invertebrates. Other workers have reported that glycine may compose as much as one-third of the total free amino acid content of the muscle tissue of crustaceans (Awapara, 1962; Florkin and Schoffeniels, 1965).

In this study, the highest glycine concentrations occurred in A. clausi (30.9%) and P. minutus (33.8%). In the marine species C. finmarchicus, glycine represented only 22.2% of the total free amino acid content. This is in agreement with the glycine value of 24.6% obtained for C. finmarchicus by Cowey and Corner (1963). Likewise, in the stenohaline lobster Homarus vulgaris, glycine represents only 21.9% of the total alpha-amino nitrogen content (Kermack et al., 1955). There appears to be a decrease in glycine, relative to the total free amino acid pool, in the strictly marine species. The exact function of the large glycine fraction, characteristic of marine crustaceans, is not yet known. But it has been suggested that glycine may have an important function in the cellular adjustment of organisms to estuarine conditions (Awapara, 1962; Florkin and Schoffeniels, 1965). Differences in the glycine concentrations of these six copepod species appear to be related to the increasing salinity of their environments (Figure 1). Thus, glycine may have a similar adaptive function in copepods.

Taurine. Taurine was not detected in terrestrial or fresh water molluscs (Simpson et al., 1959). Allen and Awapara (1960) showed that the brackish water clam Rangia cuneata had the ability to biosynthesize taurine from injected S^{35}-methionine, but that it lacked the ability to retain taurine in its tissues. The absence of taurine does not appear to be characteristic of crustaceans. Cowey (1961) reported the presence of small amounts of taurine in the muscle tissues of the fresh water crayfish

Astacus pallipes. Taurine has also been reported in the free amino acid fraction of the fresh water crab Eriocheir sinensis (Bricteaux-Grégoire et al., 1962). Likewise, in this study, the free amino acid fraction of the fresh water copepod Diaptomus sp. contained small amounts of taurine (3.7%, Table 6).

A taurine concentration of 19.2 µmoles/g wet weight (or 85.7 µmoles/g dry weight) has been reported for the marine copepod C. finmarchicus by Cowey and Corner (1963). A taurine concentration of only 35.6 µmoles/g dry weight was obtained for C. finmarchicus in this study (Table 13, Appendix I). The difference may result from the different extraction methods used. Cowey and Corner (1963) extracted their free amino acids with 80% ethanol, whereas the free amino acids in this work were extracted with a chloroform-methanol-water solvent (Bligh and Dyer, 1959). Preliminary experiments in this laboratory indicate that ethanol extracts higher quantities of all the free amino acids than the present method (Jeffries, personal communication). The possibility that the difference may be racial or environmental is not discounted. Hillman (1964) suggested that oyster populations could evolve free amino acid patterns suitable to their particular location. This may also apply to copepods.

In a study of the amino acid content of C. finmarchicus and that of its food, Cowey and Corner (1961) found that taurine was always present in the protein hydrolysates of C. finmarchicus, but that it did not occur in any of the phytoplankton samples they analyzed. This implies that C. finmarchicus can synthesize taurine either from cystine as in other animal tissues (Cowey and Corner, 1961) or from methionine as in the molluscs (Allen and Awapara, 1960). The presence of taurine in all six

copepod species seems to indicate that biomethylation occurs in the Copepoda as well as in the higher invertebrates. Furthermore, this observation may support the speculation of Awapara and Simpson (1967) that biomethylation was established early in the development of life.

Alanine. Diaptomus sp. and E. affinis differ from the other four copepods in that alanine is the predominant amino acid (Tables 5 and 6). The former is a fresh water species; the latter is a true estuarine copepod whose presence has been reported in a fresh water lake (Engel, 1962). In the marine copepods, glycine is the predominant amino acid. The only other organism in which alanine is the most abundant amino acid is Rangia cuneata, a brackish water clam (Allen, 1961). In the fresh water crustaceans, Astacus pallipes (Cowey, 1961), Astacus fluviatilis (Camien et al., 1951), and Eriocheir sinensis (Bricteaux-Grégoire et al., 1962), glycine is the predominant amino acid. However, in another fresh water crayfish, Astacus astacus, arginine is the most abundant free amino acid (Duchâteau-Bosson and Florkin, 1961).

The shift from the predominance of glycine in the marine copepods (A. tonsa, A. clausi, P. minutus, C. finmarchicus) to the predominance of alanine in the fresh water and brackish water species may imply a shift in the metabolism of amino acids (i.e., a shift in the rates of catabolism and anabolism of amino acids). According to Florkin (1964), a change at the biochemical level in the metabolism of amino acids may be partly responsible for the colonization of brackish or fresh water. Such a change could be initiated by a decrease in the inorganic ions of the medium. These, in turn, would exert their effect on the metabolism of amino acids through the enzyme systems. Differences in the rates of transamination reactions, caused by differences in the inorganic content of the environment, could explain the greater accumulation of alanine in

one group and that of glycine in the other group.

Glutamic acid. All species contained glutamic acid (Tables 5 and 6). However, on a relative basis, the highest values occurred in E. affinis (6.1%) and Diaptomus sp. (5.8%). The distribution of this amino acid is similar to that of alanine, and both are inversely related to the relative glycine distribution (Figure 4). This may further support the speculation of a shift in the metabolism of amino acids in these two species.

Aspartic acid. Aspartic acid was present in small quantities in all the copepod species (Table 5, Figure 1). The oceanic species C. finmarchicus had the highest absolute concentration (8.7 μmoles/g dry weight). Cowey and Corner (1963) found only 0.89 umoles/g wet weight (or 3.97 μmoles/g dry weight) of aspartic acid in the free amino acid fraction of C. finmarchicus. Their value is considerably lower than that reported here (Table 13, Appendix I). Differences in the extraction procedures could selectively affect this free amino acid and, thus, explain some of the variation.

Alanine, glutamic acid, and aspartic acid are closely linked to the citric acid cycle, and, as a result, their concentrations in the cells may be controlled by the reactions of this cycle (Simpson et al., 1959). Emerson (1967) showed that these three amino acids were central in the transfer of amino nitrogen in Artemia salina. Among the six copepod species, differences in the concentrations of alanine, glutamic acid, and aspartic acid may be the result of differences in their metabolism which, in turn, may be affected by such factors as salinity, temperature, and the ionic composition of the environment.

Proline. Diaptomus sp. had the lowest absolute and relative proline concentrations, P. minutus the highest (Tables 5 and 6, Figure 1). Low proline levels have been reported in other fresh water crustaceans: Astacus pallipes (Cowey, 1961) and Astacus astacus (Duchâteau-Bosson and Florkin, 1961). The relative proline levels of the marine copepod C. finmarchicus were also comparatively low (6.4%). This is surprising in view of the fact that Kermack et al. (1955) found proline to represent 37.3% of the total alpha-amino nitrogen content of the lobster Homarus vulgaris. From the results of Cowey and Corner (1963) for C. finmarchicus, a relative proline value of 8.5% was calculated which agrees well with the present results. Because the proline concentrations vary among the six copepod species, depending upon the salinity of the environment, proline may be another amino acid that is of adaptive significance.

Other amino acids. The absolute concentrations of methionine, cysteic acid, aspartic acid, valine, threonine, serine, tyrosine, leucine, isoleucine, phenylalanine, and lysine appear to increase in a linear manner with the increasing salinity of the environment (Figure 1). But because of the small number of samples, it is difficult to ascertain the exact relationship. Furthermore, the experimental error is only a rough estimate of the real error, and, thus, it compounds the difficulty in interpretation. Additional samples of P. minutus would be helpful since the free amino acid values of this species are either consistently lower or overlap with those of A. clausi. Of importance, however, is the observation that the distribution patterns of glycine, alanine, proline, taurine, glutamic acid, and arginine are distinctly different from the distribution patterns of the above-mentioned free amino acids. The former group of amino acids appear to increase in a curvilinear manner

with the increasing salinity of the environment (Figure 1).

Similar relative concentrations of cysteic acid, threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, and histidine occur in E. affinis, A. tonsa, A. clausi, and P. minutus (Table 6). Likewise, similar but higher values occur in the fresh water copepod Diaptomus sp. and the marine copepod C. finmarchicus. On the basis of the relative distribution patterns of these free amino acids, the six copepod species may be differentiated into two groups: (1) the fresh water and marine species which are restricted to their respective environments and are exposed to a constant salinity environment; and (2) the four estuarine-marine species which are exposed to a constantly fluctuating environment, with respect to salinity. Similarities in the relative concentrations within the groups and differences between the two groups may indicate differences in the modes of osmotic adjustment of these two groups to their respective environments.

B. The Curvilinear Relationship between the Major Free Amino Acids and the Optimum Salinity

In the six copepod species, glycine, proline, taurine, alanine, glutamic acid, and arginine are the predominant free amino acids (Figure 1). There is a curvilinear relationship between the concentrations of these amino acids and the midpoint of the salinity range that is optimum for the propagation of each species. Thus, these particular free amino acids may be of adaptive significance. It must be mentioned that any salinity within the optimum ranges (shown in Figure 2) can be considered as optimum for the propagation of the species. For purposes of illustration, however, the midpoint of the optimum range was selected as representative of the optimum salinity.

In higher invertebrates it has been shown that glycine, proline, taurine, alanine, and glutamic acid may be important in cellular adaptation to changing salinities (Awapara, 1962; Florkin and Schoffeniels, 1965). For a single organism Mytilus edulis, over a salinity range of 5-32 o/oo, a linear correlation was found between its taurine concentration and the sea water salinity (Lange, 1963). At low salinities, where the total free amino acid content of Mytilus was low, the mussel had reduced its relative as well as absolute amounts of taurine. In this way, through the "sparing effect" of taurine, Mytilus could maintain its essential amino acids high. A similar linear correlation between the taurine concentration and the salinity, over a range of 23-33 o/oo, was observed in Strongylocentrotus droebachiensis (Lange, 1964).

Among the copepod species, the relationship between the taurine concentration and the optimum salinity is curvilinear (Figure 1). The highest absolute amounts of taurine occur in A. clausi, but it has lower relative amounts of taurine than P. minutus and E. affinis (Tables 5 and 6). Of these three species, E. affinis is restricted by its salinity tolerance to the lower end of the salinity range, P. minutus to the higher end. A. clausi, alone, can propagate at both the higher and lower end of this salinity range. This may be related to the ability of this species to reduce its relative taurine levels while maintaining the absolute levels of taurine high. Compared to its congeneric associate, A. tonsa has reduced both its absolute and relative amounts of taurine. If taurine does have a "sparing effect" on the use of essential amino acids, as suggested by Lange (1963), the reduced levels of taurine in A. tonsa may partly explain its greater tolerance to low salinity waters.

In Rangia cuneata, the brackish water clam, Allen (1961) found a curvilinear relationship between the concentrations of alanine,

glycine, glutamic acid, aspartic acid, and the salinity, over a range of 3-25 o/oo. Maximum alanine (225.4 µmoles/g dry weight), glycine (51.6 µmoles/g dry weight), glutamic acid (32.3 µmoles/g dry weight), and aspartic acid (16.2 µmoles/g dry weight) concentrations occurred at a salinity of 17 o/oo. There was also a maximum loss of tissue water and gain of inorganic ions at salinities of 17-20 o/oo. At higher salinities, all values decreased. According to Allen (1961), a shift in osmotic control had occurred at 17 o/oo. Whether this represents an actual shift in the osmotic control mechanisms is debatable. It does indicate, however, that the free amino acids of a single species can respond to increasingly higher salinities in a curvilinear manner.

Similarly, the most concentrated free amino acids in a series of copepod species, with increasingly higher optimum salinity ranges for propagation, bear a curvilinear relationship to the optimum salinity (Figure 1). Maximum values of glycine, proline, alanine, glutamic acid and arginine occur in A. clausi and P. minutus. The former species can occur offshore, as well as in low salinity waters (see Section F, Review of Literature), and is less restricted to a particular salinity range than the other copepods. The latter species inhabits a transition zone between the oceanic and estuarine environments. Both copepods are subject to great environmental fluctuations. The maximum concentrations of these free amino acids appear to reflect the fluctuating environments of the two species.

In the strictly marine copepod C. finmarchicus, the absolute concentrations of most free amino acids appear to be higher than in P. minutus (Table 5, Figure 1). But the levels of taurine, proline, glycine, alanine, and glutamic acid appear to be reduced. The reduction

in the levels of the major free amino acids in Calanus may reflect the stability of the oceanic environment and imply a shift in osmotic control away from the free amino acids. The main problem of Calanus would appear to be ionic regulation (Potts and Parry, 1964). Thus, although a large free amino acid fraction is present in this species, the main components of this fraction may have been reduced, in accordance with the small salinity range to which the species is adapted. Unfortunately, this observation and interpretation is based on only two samples of P. minutus and one of C. finmarchicus. More samples of both species are needed to establish the exact relationship.

Acartia tonsa has lower absolute levels of glutamic acid, proline, glycine, alanine, and arginine than A. clausi (Figure 1). Of the two, it is also the most tolerant to dilution and restricted in its distribution to warm, shallow estuaries (Lance, 1963, 1964b; Jeffries, 1962b). The reduced levels of these free amino acids, and, hence, a reduced internal osmotic pressure may partly explain the greater tolerance of A. tonsa to dilution. Jeuniaux et al. (1961b) found that in two related crustaceans, Leander squilla, the more efficient osmoregulator, had lower levels of glycine, alanine, glutamic acid, and proline than Leander serratus. Their taurine and arginine levels were similar. In a similar study on annelids, these workers showed that the more euryhaline Nereis diversicolor had two times less glycine than its relative, Perinereis cultrifera (Jeuniaux et al., 1961a). Likewise, the lower levels of the major free amino acids in A. tonsa may imply that it is the more euryhaline of the two species, and, hence, a more efficient osmoregulator at the lower end of the salinity range than A. clausi.

E. affinis has higher levels of taurine, glutamic acid, proline, glycine, and alanine than Diaptomus sp., the fresh water copepod (Table 5). This condition may correspond to the increased osmotic pressure of its environment. On the other hand, E. affinis has lower concentrations of these amino acids than A. tonsa which may reflect its ability to tolerate still lower salinities than A. tonsa.

Prosser (1961) has stated that animals tend to maintain an "optimum" osmotic concentration for a given environment. The observed free amino acid concentrations of these six copepods may represent the optimum, intracellular free amino acid levels for these organisms in their particular environments.

C. Total Free Amino Acid Concentrations in Relation to the Optimum Salinity

The correlation in the six copepod species between total free amino acid concentrations and optimum salinities is, likewise, curvilinear (Figure 2). Diaptomus sp., a copepod restricted to fresh water, has the lowest concentration of total free amino acids. This condition is probably a response to the low osmotic pressure of the fresh water environment. E. affinis and A. tonsa, estuarine copepods restricted to environments in the lower end of the salinity range, have lower total concentrations than A. clausi and P. minutus, which are copepod species restricted to the higher salinity environments. Maximum total concentrations of free amino acids occur in the latter two species which are copepods that are also subject to continuous changes in the osmotic environments between the estuarine and the oceanic. Calanus, a marine species restricted to narrow salinity variations, has subsequently lower concentration levels (Table 5). These six copepod species may represent local populations whose total free amino acid levels are adapted to the

particular salinity range of their respective environments. Adaptive mechanisms have been noted for other organisms in estuarine habitats. Since all estuaries differ in their salinity structure and range, Hillman (1964) suggested that local oyster populations could evolve control mechanisms suited to their particular location. Korringa (1952), further, implied the possible existence of physiological races of oysters related to salinity.

The six copepod species studied represent three distinctly different habitats: (1) the fresh water pond, (2) the estuary, and (3) the open ocean. The copepods in each environment face different problems of adjustment which should elicit physiological responses of adaptation, peculiar to that particular environment. Within one environment, the different copepod species may respond uniquely to the demands of that environment (Prosser, 1961). In addition, the response of each species to its environmental demands may be influenced by its degree of adaptive evolution to the environment.

The osmotic problem faced by a fresh water organism such as Diaptomus sp. must be one of exclusion of water and the absorption of salt from a medium with a salt content as low as 0.01-0.001% that of sea water (Robertson, 1960). The possession of a mechanism for the maintenance of a higher blood concentration than the medium ("anisosmotic extracellular regulation") must be of greater importance than reliance on cellular toleration to dilution ("intracellular isosmotic regulation"). The reliance of Diaptomus sp. on the former osmotic control mechanism rather than on the latter may be reflected in its low total free amino acid levels, and especially in the low concentrations of glycine, taurine, and proline. However, a shift in the metabolism of amino acids responsible for the predominance of alanine in this species, along with a

reduction of its free amino acids, may have enabled Diaptomus sp. to successfully invade fresh water.

For the estuarine copepods, E. affinis, A. tonsa, A. clausi, and P. minutus, the problem is one of adjustment to salinity fluctuations. These species have wide salinity tolerances and distribution ranges (Section F, Review of Literature). However, the differentiating factor is the salinity that supports the growth of abundant populations. This restricts E. affinis to a salinity range of 5-15 o/oo for propagation; the Acartia species, to a range of 10-32 o/oo; and P. minutus, to salinities over 30 o/oo (Jeffries, 1967). The total free amino acid concentrations of these four species may represent not only an adaptation to a specific salinity range but also the degree of selective or adaptive evolution to the salinity range of certain environments. Of the four species, E. affinis has the lowest total free amino acid levels. The organism's free amino acid distribution, characterized by the predominance of alanine, is very similar to that of the fresh water copepod Diaptomus (Table 5). Ceccaldi and Daumas (1967) found that among siphonophores the lowest free amino acid concentrations were characteristic of the most highly evolved organisms, the highest of the most primitive. If this applies to other organisms, then the low free amino acid levels in E. affinis may also be related to the length of time this species has been exposed to brackish water.

According to Jeffries (1967), combinations such as the Acartia species may represent the remains of an evolutionary pattern that spread from warm water into temperate estuaries. Because A. tonsa has a greater tolerance to dilution and is a warm acclimated species, he feels that it has been exposed to brackish water for longer time periods than A. clausi. The lower free amino acid concentration of A. tonsa may support this spec-

ulation.

The lower total free amino acid levels of A. tonsa, furthermore, indicate that it is a more efficient osmo-regulator than its congener. Other physiological studies support the hypothesis that A. tonsa is an osmoregulator (Lance, 1965). For instance, at low salinities, its respiratory rate is increased, a possible indication that work is being done to maintain an osmotic equilibrium. However, Lance (1965) feels that adjustment and tolerance at the cellular level will not largely influence the extent to which this species penetrates into brackish water. Instead, she feels that the ionic regulatory processes must be of greater importance. But by lessening the amount of work required of the ionic regulatory mechanism, such tolerance and adjustment at the cellular level may partly influence the extent to which this species invades brackish water.

On the other hand, the high total free amino acid levels of A. clausi may indicate that: (1) this species has been exposed to brackish water for a shorter time span than its congener; and/or (2) this species is an osmoconformer rather than an osmoregulator. If indeed A. clausi is an osmoconformer (an organism whose body fluid concentration changes with the medium), then its survival in the estuary would depend largely on its ability to tolerate a greater range of internal variation (Prosser, 1964). Hence, the mechanism of "intracellular isosmotic regulation", involving the free amino acids, would be of prime importance in its survival. The high total free amino acid concentration of P. minutus may, likewise, indicate that this species is an osmo-conformer. Unfortunately, data from other physiological studies do not exist to support these speculations.

For Calanus, restricted to the marine environment, the main problem must be that of ionic regulation and water absorption. The exact

opposite problem is encountered by the fresh water copepod Diaptomus sp. The relative free amino acid distributions of the two species indicate similarities in all free amino acid levels, with the exception of taurine, glutamic acid, proline, glycine, and alanine (Table 6). That Calanus finmarchicus may possess a mechanism for "anisosmotic extracellular regulation" is indicated by the presence of a pair of excretory maxillary glands which have not been found in other copepods. These glands may be involved in ionic regulation (Lance, 1965). This may partly explain the lower total free amino acid levels and, especially, the lower levels of taurine, glutamic acid, proline, glycine, and alanine that were observed in this species.

Other variables which have not been evaluated in the present study but which could influence the observed free amino acid concentrations of the copepod species studied include: food, temperature, state of maturity, starvation, the ionic composition of the environment, and pollution. The past histories of the copepod species are unknown, and the analysis is based on a small number of samples. Furthermore, large variations were observed not only in total free amino acid concentrations but also in the concentrations of individual amino acids within samples of the same species. The results must, therefore, be interpreted with reservation and with regard to the possible modifying influences of the above-mentioned variables. The adaptation of free amino acid concentrations to the particular salinity range of specific environments, which was indicated by the curvilinear relationship between free amino acid levels and the optimum salinities of the six copepod species, remains to be observed in other organisms.

V. SUMMARY

1. The six copepod species, Diaptomus sp., Eurytemora affinis, Acartia tonsa, Acartia clausi, Pseudocalanus minutus, and Calanus finmarchicus, collectively represent a chain of species populations that span a salinity gradient from fresh water to the open ocean. Each member of this chain has a higher optimum salinity for propagation.
2. The qualitative free amino acid compositions of these six species are similar. All contain 19 common free amino acids, with the exception of E. affinis, which has no free histidine.
3. Major differences occur in the concentrations of individual amino acids and in the total free amino acid levels. Diaptomus sp. and E. affinis are characterized by the predominance of alanine; glycine is predominant in the estuarine-marine species.
4. The most abundant free amino acids in the fresh water Diaptomus sp. are alanine, threonine, and the basic amino acids, arginine and lysine. Glycine, taurine, proline, arginine, alanine, and glutamic acid are the most abundant free amino acids in the other five species.
5. The concentrations of glycine, proline, taurine, glutamic acid, alanine, and arginine bear a curvilinear relationship to the optimum salinity. Maximum concentrations occur in A. clausi and P. minutus; the concentration levels appear to be reduced in the marine copepod C. finmarchicus.
6. The concentrations of the remaining 13 free amino acids may bear a linear relationship to the optimum salinity.

7. The total free amino acids also show a curvilinear relationship to the increasing optimum salinities of the six species. Although the fresh water Diaptomus sp. has lower total free amino acids than the marine copepod C. finmarchicus, the highest total free amino acid concentrations occur in A. clausi and P. minutus. Lower levels occur in the more euryhaline species, E. affinis and A. tonsa.
8. The ecological significance of the curvilinear relationship is considered in terms of: (1) adaptation to a specific salinity range, in accordance with the osmoregulatory demands of the particular environment; (2) time period of adaptation to the respective environment; and (3) other variables such as food, temperature, starvation, state of maturity, the ionic composition of the environment, and pollution which have not been evaluated in the present study.

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APPENDIX I

Table 8. Free amino acid concentrations of replicate analyses on a single sample of Diaptomus obtained from Wordens Pond, Rhode Island on August, 1967. Values expressed as μmoles/g dry weight and per cent of total free amino acid pool.

AMINO ACID	REPLICATE I μmoles/g	PER CENT %	REPLICATE II μmoles/g	PER CENT %	MEAN μmoles/g	MEAN PER CENT %
CYS	1.1	1.5	1.4	1.5	1.2	1.5
TAU	2.6	3.4	3.4	3.9	3.0	3.7
ASP	1.9	2.6	2.9	3.3	2.4	3.0
THR	5.2	7.0	5.8	6.6	5.5	6.7
SER	3.7	5.0	4.4	5.0	4.0	5.0
GLU	4.5	6.1	5.0	5.7	4.7	5.8
PRO	3.0	4.0	3.5	4.0	3.2	4.0
GLY	4.2	5.6	5.1	5.8	4.6	5.7
ALA	11.5	15.5	13.4	15.2	12.4	15.4
CYST	0.5	0.7	0.9	1.0	0.7	0.9
VAL	3.6	4.9	4.2	4.8	3.9	4.8
METH	1.1	1.5	1.5	1.7	1.3	1.6
ILEU	2.3	3.1	2.7	3.0	2.5	3.1
LEU	4.4	5.9	5.1	5.8	4.7	5.8
TYR	2.8	3.7	3.0	3.5	2.9	3.6
PHE	2.1	2.8	2.3	2.6	2.2	2.7
ORN	0.5	0.7	0.5	0.6	0.5	0.6
LYS	6.5	8.7	7.8	8.8	7.1	8.8
HIS	1.8	2.4	2.2	2.5	2.0	2.5
ARG	10.9	14.8	13.2	15.0	12.1	14.9
TOTAL	74.2		88.3		80.9	

Table 9. The free amino acid concentration of Eurytemora affinis obtained from Pettaquamscutt River, Rhode Island, February-March, 1967. Values expressed as μmoles/g dry weight and per cent of total free amino acid pool.

AMINO ACID	Feb. 9 μmoles/g	PER CENT %	Mar. 8 μmoles/g	PER CENT %	MEAN μmoles/g	MEAN PER CENT %
CYS	2.4	1.1	1.7	0.9	2.1	1.0
TAU	30.8	14.1	27.8	13.8	29.3	14.0
ASP	3.0	1.4	1.5	0.7	2.2	1.1
THR	4.9	2.2	6.8	3.4	5.8	2.8
SER	6.0	2.8	7.8	3.9	6.9	3.3
GLU	14.3	6.6	11.4	5.6	12.9	6.1
PRO	30.0	13.8	25.5	12.6	27.8	13.2
GLY	23.2	10.7	16.7	8.3	20.0	9.5
ALA	45.3	20.8	36.9	18.3	41.1	19.6
VAL	4.6	2.1	5.9	2.9	5.3	2.5
METH	2.6	1.2	3.5	1.7	3.0	1.4
ILEU	3.4	1.6	4.4	2.2	3.9	1.9
LEU	5.0	2.3	7.5	3.7	6.3	3.0
TYR	3.4	1.6	3.3	1.6	3.4	1.6
PHE	4.4	2.0	3.4	1.7	3.9	1.9
ORN	0.9	0.4	1.7	0.8	1.3	0.6
LYS	7.3	3.3	9.9	4.9	8.6	4.1
HIS	---	---	---	---	---	---
ARG	26.1	12.0	26.2	13.0	26.2	12.5
TOTAL	217.6		201.9		210.0	
N*	1		2			

*Number of replicates

Table 10. The free amino acid concentration of Acartia tonsa obtained from Narragansett Bay, Rhode Island October, 1965 - October, 1966. Values expressed as μmoles/g dry weight and per cent of total free amino acid pool.

AMINO ACID	Oct 6[1] μM/g	Per Cent %	Nov 3[1] μM/g	Per Cent %	Aug 17 μM/g	Per Cent %	Oct 6[11] μM/g	Per Cent %	Oct 11 μM/g	Per Cent %	Oct 18 μM/g	Per Cent %	Grand Mean	Mean Per Cent
CYS	3.1	0.5	1.1	0.4	2.0	0.6	1.9	0.5	1.7	0.5	1.8	0.3	2.0	0.5
TAU	81.8	12.6	48.1	16.3	36.2	10.6	37.5	9.4	41.1	11.7	53.6	9.9	49.7	11.5
ASP	2.3	0.4	1.5	0.5	3.1	0.9	7.0	1.7	3.3	0.9	6.9	1.3	4.0	0.9
THR	9.0	1.4	4.0	1.3	6.7	2.0	13.1	3.3	8.0	2.3	16.7	3.1	9.6	2.2
SER	10.4	1.6	4.2	1.4	8.1	2.4	11.1	2.8	7.0	2.0	15.3	2.8	9.3	2.2
GLU	16.9	2.6	9.3	3.2	8.0	2.4	14.1	3.5	9.4	2.7	18.1	3.3	12.6	2.9
PRO	124.7	19.2	58.2	19.7	28.9	8.5	67.8	17.0	83.4	23.7	109.4	20.1	78.7	18.3
GLY	202.0	31.1	102.4	34.6	133.5	39.2	80.5	20.1	73.6	20.9	117.1	21.5	118.2	27.4
ALA	79.0	12.2	14.1	4.8	38.0	11.2	45.5	11.4	36.0	10.2	59.6	11.0	45.4	10.5
VAL	9.1	1.4	4.1	1.4	6.9	2.0	20.5	5.1	7.2	2.0	13.7	2.5	10.3	2.4
METH	4.4	0.7	1.5	0.5	3.5	1.0	5.6	1.4	4.2	1.2	7.1	1.3	4.4	1.0
ILEU	6.2	0.9	2.4	0.8	5.1	1.5	8.7	2.2	5.7	1.6	10.1	1.9	6.4	1.5
LEU	10.1	1.5	4.9	1.7	10.1	3.0	15.6	3.9	10.0	2.8	18.7	3.4	11.6	2.7
TYR	5.4	0.8	1.9	0.6	4.0	1.2	5.3	1.3	4.7	1.3	6.9	1.3	4.7	1.1
PHE	5.1	0.8	2.1	0.7	4.5	1.3	6.1	1.5	4.8	1.4	7.5	1.4	5.0	1.2
ORN	----	----	----	----	----	----	0.1	+	----	----	0.8	0.1	+	+
LYS	13.4	2.1	4.9	1.7	12.0	3.5	21.0	5.2	13.1	3.7	26.2	4.8	15.1	3.5
HIS	3.3	0.5	1.1	0.4	----	----	2.9	0.7	2.6	0.7	4.5	0.8	2.9	0.7
ARG	63.8	9.8	30.0	10.1	30.1	8.8	35.5	8.9	36.3	10.3	50.2	9.2	41.0	9.5
TOTAL	650.0		295.8		340.7		399.8		352.1		544.2		430.9	
N*	1		1		2		1		2		5			

*Number of replicates.

[1]Data provided by H. P. Jeffries. Samples obtained in 1965.

[11]October samples obtained in 1966.

Table 11. The free amino acid concentration of Acartia clausi from Narragansett Bay, Rhode Island, December, 1965 - April, 1966. Values expressed as μmoles/g dry weight and per cent of total free amino acid pool.

AMINO ACID	Dec 1[1] μM/g	Per Cent %	Dec 29[1] μM/g	Per Cent %	Apr 20[1] μM/g	Per Cent %	Grand Mean μM/g	Mean Per Cent %
CYS	3.5	0.5	1.8	0.3	4.5	0.8	3.3	0.5
TAU	91.9	13.2	93.2	15.2	74.2	12.5	86.4	13.6
ASP	4.3	0.6	4.9	0.8	8.0	1.3	5.8	0.9
THR	13.8	2.0	9.6	1.6	17.3	2.9	13.6	2.1
SER	10.3	1.5	6.6	1.1	15.2	2.6	10.7	1.7
GLU	16.1	2.3	22.2	3.6	12.8	2.1	17.0	2.7
PRO	104.6	15.0	98.3	16.0	92.8	15.6	98.6	15.5
GLY	233.8	33.6	199.7	32.5	154.6	26.0	196.0	30.9
ALA	68.6	9.9	62.2	10.1	66.7	11.2	65.8	10.4
VAL	16.7	2.4	11.7	1.9	15.1	2.5	14.5	2.3
METH	5.8	0.8	3.8	0.6	7.0	1.2	5.5	0.9
ILEU	10.4	1.5	7.6	1.2	10.1	1.7	9.4	1.5
LEU	16.9	2.4	12.3	2.0	18.1	3.0	15.8	2.5
TYR	7.5	1.1	5.0	0.8	8.8	1.5	7.1	1.1
PHE	7.6	1.1	6.6	1.1	9.7	1.6	7.9	1.2
ORN	+	+	+	+	+	+	+	+
LYS	18.7	2.7	12.7	2.1	23.7	4.0	18.4	2.9
HIS	5.3	0.8	3.8	0.6	3.9	0.7	4.3	0.7
ARG	60.2	8.6	52.9	8.6	52.8	8.9	55.3	8.7
TOTAL	695.8		614.9		595.3		635.4	
N*	1		1		1			

*N is the number of replicates.

[1]Data provided by H. P. Jeffries.

Table 12. The free amino acid concentration of Pseudocalanus minutus from Rhode Island Sound, December, 1965 - January, 1966. Values expressed as μmoles/g dry weight and per cent of total free amino acid pool.

AMINO ACID	Dec. 29[1] μmoles/g	PER CENT %	Jan. 26[1] μmoles/g	PER CENT %	MEAN μmoles/g	MEAN PER CENT %
CYS	2.5	0.4	1.6	0.4	2.1	0.4
TAU	106.7	15.5	60.8	14.5	83.7	15.1
ASP	3.6	0.5	4.1	1.0	3.8	0.7
THR	13.8	2.0	10.8	2.6	12.3	2.2
SER	8.6	1.2	9.1	2.2	8.8	1.6
GLU	10.8	1.6	9.5	2.3	10.1	1.8
PRO	188.8	27.5	30.1	7.2	109.4	19.8
GLY	225.1	32.8	148.9	35.4	187.0	33.8
ALA	40.2	5.9	41.6	9.9	40.9	7.4
VAL	9.8	1.4	11.5	2.7	10.6	1.9
METH	3.5	0.5	4.4	1.1	4.0	0.7
ILEU	6.4	0.9	7.4	1.8	6.9	1.2
LEU	10.6	1.5	13.7	3.2	12.1	2.2
TYR	6.2	0.9	6.5	1.5	6.3	1.1
PHE	4.8	0.7	5.5	1.3	5.1	0.9
LYS	13.4	1.9	15.5	3.7	14.5	2.6
HIS	3.9	0.6	4.1	1.0	4.0	0.7
ARG	28.6	4.2	35.8	8.5	32.2	5.8
TOTAL	687.3		420.9		553.8	
N*	1		1			

*Number of replicates

[1]Data provided by H. P. Jeffries

Table 13. A comparison of the free amino acid concentration of Calanus finmarchicus obtained 10 miles north of Provincetown, Massachusetts, August, 1967 and of C. finmarchicus from the sea of the Firth of Clyde, England. Values expressed as μmoles/g dry weight and per cent of total free amino acid pool.

AMINO ACID	Firth of Clyde[a] μmoles/g	Per Cent %	Provincetown[b] μmoles/g	Per Cent %
CYS	-----	-----	3.3	0.9
TAU	85.7	17.6	35.6	10.2
ASP	4.0	0.8	8.7	2.9
THR	9.5	2.0	13.0	3.7
SER	13.8	2.8	11.3	3.8
GLU	14.3	2.9	6.5	1.9
PRO	41.5	8.5	22.3	6.4
GLY	119.7	24.6	77.8	22.2
ALA	41.5	8.5	36.2	10.3
VAL	13.6	2.8	15.6	4.5
METH	6.3	1.3	6.2	1.8
ILEU	11.6	2.4	10.9	3.1
LEU	16.6	3.4	19.0	5.4
TYR	10.6	2.2	8.1	2.3
PHE	7.5	1.5	7.4	2.1
ORN	-----	-----	1.2	0.3
LYS	17.4	3.6	16.3	4.7
HIS	4.7	1.0	3.6	1.0
ARG	68.8	14.1	47.5	13.6
TOTAL	487.1		350.5	
N*			3	

*Number of replicates.

[a]Wet weight values of Cowey and Corner (1963) converted to μmoles/g dry weight. Free amino acids extracted with 80% ethanol.

[b]Free amino acids extracted with chloroform-methanol-water (2:1:0.8).

APPENDIX II

Table 14. Salinity data for Narragansett Bay and Rhode Island Sound, April, 1965 - October, 1966. Nar. B. (Narragansett Bay), RIS (Rhode Island Sound). *(Data provided by H. P. Jeffries), [1](Data provided by H. Russell).

Station	Location	Coordinates	Date	Mean Surface Salinity, ‰	Mean Bottom Salinity, ‰
I	Nar. B.*	41°34'13"N 71°23'30"W	Oct. 6	31.902	31.879
I	Nar. B.*		Nov. 3	31.918	31.891
I	Nar. B.*		Dec. 1	31.535	31.659
I	Nar. B.*		Dec. 29	31.311	31.272
I	Nar. B.*		Jan. 26	30.521	30.571
I	Nar. B.*		Apr. 20	30.367	30.521
I	Nar. B.*		Aug. 17	31.830	----
I	Nar. B.		Oct. 6	31.850	----
I	Nar. B.		Oct. 11	31.690	----
I	Nar. B.		Oct. 18	31.800	----
VI	Nar. B.[1]	41°26'40"N 71°24'45"W	Apr.-Dec.	31.700	32.050
VI	Nar. B.[1]		Jan.-July	31.270	32.020
VII	Nar. B.[1]	41°29'30"N 71°24'45"W	Apr.-Dec.	31.460	31.720
VII	Nar. B.[1]		Jan.-July	31.060	31.590
II	RIS*	41°20'30"N 71°20'00"W	Oct. 6	32.293	32.568
II	RIS*		Nov. 3	32.356	32.522
II	RIS*		Dec. 1	32.444	32.363
II	RIS*		Dec. 29	32.270	32.317
II	RIS*		Jan. 26	32.157	32.239
II	RIS*		Apr. 20	31.176	32.378
I	RIS[1]	41°07'10"N 71°19'35"W	Jan.-July	32.230	32.570
II	RIS[1]	41°12'00"N 71°21'20"W	Apr.-Dec.	32.170	32.400
II	RIS[1]		Jan.-July	32.200	32.510
III	RIS[1]	41°16'50"N 71°23'00"W	Apr.-Dec.	32.070	32.370
III	RIS[1]		Jan.-July	32.130	32.450
IV	RIS[1]	41°21'40"N 71°24'45"W	Apr.-Dec.	32.020	32.310
IV	RIS[1]		Jan.-July	32.000	32.360
V	RIS[1]	41°24'10"N 71°24'45"W	Apr.-Dec.	31.940	32.260
V	RIS[1]		Jan.-July	31.820	32.310

Table 15. Salinity data for Pettaquamscutt River, March, 1961 - January, 1968. Pett. R. (Pettaquamscutt River). *(Data provided by H. P. Jeffries), [a](Data provided by A. Gaines).

Depth (feet)		1	5	10	15	20	30	50
Station	Location	o/oo	o/oo	o/oo	o/oo	o/oo	o/oo	o/oo
A_2 (Mar. 13)*	Upper Pond	6.4	11.8	18.0	----	19.0	20.4	----
C_2		13.0	15.0	----	23.8	----	27.2	27.4
D_2	Lower Pond	12.0	15.3	21.8	----	----	----	----
S_5		12.0	12.0	12.0	----	----	----	----
A_2 (Mar. 23)*	Upper Pond	13.4	15.0	18.6	18.8	19.4	20.2	----
C_2		12.8	15.4	20.3	----	26.3	----	27.3
D_2	Lower Pond	12.0	15.3	19.0	25.3	----	----	----
S_5		17.4	17.4	17.5	----	----	----	----
I (Jan. 13)[a]	Upper Pond	11.40	13.98	15.01	15.54	15.54	21.44[1]	----
I (Feb. 9)	Bridgetown Bridge	19.3	----	----	----	----	----	----
I (Mar. 8)		5.9	----	----	----	----	----	----

[1]Value obtained at 25 feet.