OSMOTIC AND IONIC REGULATION

IN CRUSTACEA

by

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Preface

This paper is an introduction to the processes of
osmoregulation and ionic regulation in Crustacea, and
a selected review of the literature pertinent to a study
of those processes. The problems of adaptation to the
environment and flexibility in the face of changing en-
vironmental influences are basic to an understanding of
the evolution of morphological and physiological charac-
teristics. This paper will attempt to elucidate the role
of regulation of ions and water in facilitating ecological
flexibility, and the limits imposed on creatures that
lack this ability.

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Every animal is inescapably in some form of contact
with his environment, and the interaction between that
environment and the animal's body fluids is a matter of
crucial importance. Life began in water, and as living
creatures are composed, on the average, of 70-85% water
(Nicol, 1960), direct exposure to air calls for relatively
sophisticated mechanisms to avoid partial dessication.
Terrestrial animals must therefore be equipped with a
means to conserve water, and even marine forms often must

work to retain water although they actually live immersed
in it. If the concentration of ions in the body fluids
of a marine animal is less than the concentration of ions
in sea water, water may move out of the animal by osmosis.
The result may be that the animal is in need of water to
dilute its body fluids to a concentration which will allow
normal physiological processes to continue. Conversely,
fresh-water animals have the problem of maintaining a fin-
ite amount of water in their bodies. Since the medium is
less concentrated than their body fluids, ions may diffuse
out into the medium, and water may enter such animals by
osmosis. They then have the problem of preventing too
great a dilution of their internal ionic concentration.
Thus every animal, no matter where it lives, must cope
with the stresses of a basically hostile world.

It is not enough to consider the problem solved if
an animal does maintain an optimum amount of water in its
body; that is, the entire problem is not to achieve a set
concentration. Certain ions are required, and the body
must have them in definite proportions. If the external
medium does not offer such ions in the concentrations nec-
essary, an animal must find some means to selectively with-
draw them from the medium; without such mechanisms the
animal must assume the concentration for each ion as it
is represented in the medium, and often this value is not
within the limits set for an optimum balance of chemicals.

Some Useful Terms

Every water medium has a certain salinity, or concentration of salt, which may be expressed in parts per thousand (‰). Thus sea water has a salinity of approximately 34.5 ‰, while fresh water has a salinity of less than .5‰ (Prosser and Brown, 1950). Physiologists label animals which are able to cope with a wide range of environmental salinities as euryhaline, and those which can exist successfully in only a narrow range as stenohaline. Many animals passively adopt the osmotic concentration of the medium. Such animals are termed osmoconformers, or poikilosmotic; if, instead, an animal maintains a fairly constant internal concentration, despite the concentration of the medium, it is termed an osmoregulator, or homoiosmotic.

Osmoregulation refers to regulation of the total particle concentration of the body fluids at levels which may differ from the concentration of the medium. A solution which is more dilute than another is hyposmotic relative to that solution; if it is more concentrated, it is hyperosmotic. When, instead, water is balanced between two systems, they are isosmotic.

Ionic regulation is "the maintenance in a body fluid of concentrations of ions differing from those in passive equilibrium with the external medium." (Robertson, 1953). It is useful to define regulation, either osmotic or ionic,

as an active, energy-requiring process. At the expense of
such energy a successful osmotic and ionic regulator is
expected to be able to survive in salinities far different
from its internal milieu, while osmoconformers and ion
conformers can never leave the medium which approaches their
own optimum concentration of ions.

General Principles

A membrane which is permeable to water molecules but
not to solute particles is termed a semipermeable membrane.
If such a membrane separates two solutions of different
concentrations, water will move into the more concentrated
solution until the two solutions have the same molal con-
centration.(Molal = moles of solute/kg water.) This move-
ment of water is osmosis (Potts and Parry, 1964). An equa-
tion for osmosis of water into a body of concentration C2
from a medium of lower concentration C1 is given by
$v_w = k_w (C2-C1)$, where v_w is the rate of water movement
and k_w is a rate constant (the permeability coefficient
of the external body surface to the water) (Croghan, 1961).

Diffusion is the process of random mixing of solute
particles in a solution of unequal particle distribution
until an even distribution is reached. There is a net
movement of particles from a place of greater concentration
to a place of lesser concentration (Potts and Parry, 1964).
Two solutions, of concentrations C1 and C2, separated by

a semipermeable membrane of thickness T, constitute a concentration gradient of $C2-C1/T$. The rate of diffusion across the membrane in an area A is DA $C2-C1/T$, where D is a diffusion constant (Potts and Parry, 1964). For a body in a medium less concentrated than itself, the equation for solute lost from the body by diffusion is $r_d = k_s (C2-C1)$, where r_d is the rate of solute diffusion, k_s is a rate constant (the permeability coefficient of the external surface to the solute), C2 is the body fluid concentration, and C1 is the medium concentration (Croghan, 1961).

If there is a potential difference between two solutions due to the net charge of their respective solute particles, then there is a potential gradient between the solutions, and charged particles will tend to move down the gradient and thereby decrease the potential difference. A potential gradient may either reinforce or inhibit diffusion due to a concentration gradient, depending upon the direction of the gradients.

The Energetics of Osmotic and Ionic Regulation

When molecules move down a concentration gradient, energy is liberated. To move a solute against a gradient requires energy. The work, W, required to move 1 mole of solute from a solution of molal concentration C1 to a solution of higher concentration C2 is $W = R T \ln (C2/C1)$

(Potts and Parry, 1964). If there is also a potential
difference of E volts between the solutions, there is
an energy requirement of zEF to move 1 mole of ion
against the electrochemical gradient, where z is the
valency of the ion and F the Faraday. Then the equa-
tion for work is $W = R T \ln (C1/C2) + zEF$ (Potts and
Parry, 1964).

If uptake proceeds from a concentration C1 to a
concentration C2 by carrier sites with a high affinity
for solute particles in C1, the rate of transport from
C1 to C2 is determined by the number of sites and is
independent of the concentration of solute in C1. (The
carrier site is saturated at very low concentrations of
solute in C1.) Croghan (1961) says that if one assumes
that the activation energy of transport is high, then
the system must operate well away from the equilibrium
position to maintain an appreciable rate. Therefore, he
states, the transport system is probably unidirectional
and the rate of transport by a given number of sites is,
for the most part, independent of the body fluid concen-
tration. Then r_m, the rate of active uptake from the
medium, $= k_m n_m$, and r_t, the rate of active uptake from the
excretory tubule, $= k_t n_t$, where k_m and k_t are the rate
constants of the carrier mechanisms and n_m and n_t are the
numbers of active carrier sites (Croghan, 1961). Shaw

(1958), in a study of Na absorption in the crayfish
Astacus pallipes, verified that in a medium of low con-
centration the rate of active uptake is proportional to
the concentration of the medium. The concentration de-
termines the number of ions coming into contact with
transport sites. At high concentrations of the medium
all transport sites are saturated, and absorption of ions
is independent of the concentration. Given the same per-
meability and blood concentration, the energy cost of
osmoregulation is then dependent on the surface to volume
ratios of the animal. A small animal will require more
work per unit mass of body tissue than a large animal to
maintain a certain blood concentration. A decreased
blood/medium concentration gradient will lessen the work,
and a smaller animal usually maintains for this reason a
lower blood concentration than does a larger animal living
in the same medium (Lockwood, 1962).

When water is balanced, $v_w = v_u$, where v_u is the rate
of urine production. Solute will then enter the excretory
organ at a rate, $v_f, = k_w\ C2\ (C2-C1)$ and solute will be
lost from the animal via the urine at a rate $v_u = k_w\ C_u\ (C2-C1)$
where C_u is the concentration of the urine excreted (Croghan,
1961). The rate of active uptake from the excretory tubule
is $r_t = k_w\ (C2-C_u)(C2-C1)$. The rate of active uptake from
the medium is $r_m = k_s\ (C2-C1) + k_w\ C_u\ (C2-C1)$. When C1 is

small compared to C2, $C2 = \sqrt{r_m + r_t / k_w}$ (Croghan, 1961).
Therefore the concentration of body fluid in a given med-
ium concentration is determined by the permeability co-
efficients and the rates of the uptake mechanisms.

Fresh-water Crustacea

Crustaceans in fresh water must keep their body
fluids hyper-osmotic to the medium because they would
have body fluids too dilute for cellular functions if
they were isosmotic to the fresh water. Water is con-
tinually entering osmotically, and ions leaving their
bodies through excretion and diffusion. Because it re-
quires energy to maintain an internal concentration against
such a gradient, fresh-water animals have found it useful
to acquire a toleration to a less concentrated internal
milieu than many marine organisms. The latter are often is-
osmotic to sea water and do not have as strong a gradient
(Robertson, 1960). Since fresh-water does not have the same
proportion of ions as required in body fluids, fresh-water
forms must regulate ionically as well as osmotically.

To maintain themselves in fresh water, crustaceans
may use essentially three mechanisms: 1) a low permeabil-
ity to water and salts, 2) a selective uptake of ions from
the medium at the body surface or from the gut, and reab-
sorption from the urine, and 3) elimination of water by
production of a dilute urine.

The amphipod genus _Gammarus_ has representatives
living in a wide range of environmental conditions.
Gammarus pulex is a fresh-water form (Shaw, 1961c). It
has been suggested that its mechanisms for regulation
may in fact be characteristic of fresh-water Crustacea
in general. The mechanism it uses for ionic uptake has
a high affinity for Na ions, and maximum transport is
reached at external concentrations of near 1mM/l. Further-
more, the rate of uptake is increased by only a small drop
in the internal Na concentration. The adaptive signif-
icance of these facts is better appreciated by comparing
this organism with its brackish-water relative, _Gammarus
duebeni_. _G. duebeni_ presents a low affinity for Na ions,
and its mechanism does not reach maximum transport of ions
until the external concentration is about 10 mM/l. At a
medium concentration of .1mM/l, for instance, _G. pulex_
would be capable of transporting about three times as
many ions as transported by _G. duebeni_ at the same exter-
nal concentration. It is clearly a better regulator than
G. duebeni in mediums of very low concentrations.

Werntz (1963) showed that activation of the regula-
tory mechanism in gammarids is gradual, with the critical
concentration (the concentration below which maximum reg-
ulation rate is employed) being higher in marine species
than in fresh-water species. There has been some disagree-

ment as to whether the degree of activation actually depends on the concentration of the external medium or of the blood, but Shaw (1959) favors the former.

Osmotic regulation in four species of gammarids was compared by Werntz (1963). He found that the fresh-water species G. fasciatus was able to regulate its blood concentration at a very low level of external salinities, while the brackish and marine forms were able to regulate only at intermediate and high salinities, respectively. Measured by freezing point depression, G. fasciatus maintained a blood concentration of 0.35-0.50 molal% in equivilants of ideal solute, the brackish-water G. tigrinus kept its blood at 0.55-0.65 molal%, and the marine forms G. oceanicus and Marinogammarus finmarchicus maintained concentrations of 0.65-0.85 molal%. This data indicates the toleration by fresh-water forms of more dilute body fluids than maintained by animals living in waters of higher salinity.

There is also evidence in the gammarids for a decreased permeability in the fresh-water animals relative to animals living in more saline waters. Werntz (1963) found that the rate of urine flow in these animals is directly proportional to the osmotic gradient between the blood and the medium, and it is therefore evident that osmotically absorbed water is eliminated as urine. The rate of flow of urine in the

marine G. oceanicus is 10.5% (% of body weight per hour
per molal gradient), but in G. fasciatus it is only 5.9%.
It is clear that G. fasciatus is less permeable to water
than is G. oceanicus. Magnin (1964) has verified that
decreased permeability is correlated with adaptation to
fresh water.

G. fasciatus reduces salt loss, not only by having
a reduced permeability, but also by producing a urine
that is hypotonic* to its blood. G. oceanicus produces
urine that is isotonic to its blood, and has a higher
rate of loss of urinary salt (Werntz, 1963). Lockwood
(1961) has shown that when G. duebeni is transferred
from 100-175% sea water to fresh water, its urine changes
from isotonic to hypotonic. This ability to rapidly
change the concentration of the urine and thereby regulate
salt loss is an important adaptation to an environment
subject to changing salinity, such as pools which may be
suddenly inundated with salt or fresh water.

W.T.W. Potts (1954) has done experiments concerning
the energy requirements of osmoregulation in fresh water.
He lists the factors which affect this requirement as
1) the animal's permeability, 2) the surface area, 3) the
concentration of the medium, and 4) the urine and blood
concentrations.

Considering the last factor, one finds that most

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*"Although many authors use the terms isotonic as synonymous with
isosmotic (and similarly, hypertonic and hypotonic), the terms are
not identical. Tonicity is defined in terms of the response of
cells immersed in a solution. A solution is said to be isotonic
with a cell (or tissue) if the cell neither swells nor shrinks
when immersed in it." (Potts and Parry, 1964).

fresh-water Crustacea, like G. fasciatus, produce a urine
hypotonic to their blood. Eriocheir sinensis, a fresh-
water grapsoid crab which returns to the sea to breed, is
one of the exceptions since it produces a urine isosmotic
to its blood at all external dilutions (Robertson, 1957).
Potts uses this crab as an example in support of the
thermodynamic advantages to be derived from producing
blood-hypotonic urine.

His reasoning proceeds like this: If an animal pro-
duces V l. of urine/hr. of a concentration U moles/l., it
is losing VU moles of solute/hr. To balance this loss the
animal must absorb solute from the medium. The work, W,
required to transfer 1 mole of solute from concentration M
to concentration $B = R \, T \ln B/M$ cal. Then the minimum
work to balance solute loss = $R \, T \, V \, U \ln B/M$ cal/hr. Potts
assumed the following values for a 60 gm. crab: $M = 0.006$
mole/l., $B = 0.320$ mole/l., $U = 0.320$ mole/l., $V = 1.04 \times 10^{-4}$ l./hr., metabolic energy = 14 cal./hr. He calculated
that $V = P \, A \, (B-M)$ where P is the permeability and A is
the area. By this equation, $P \, A = 3.312 \;\; 10^{-4}$ l./hr. By
$R \, T \, U \, V \ln B/M = W$, $W = 0.0757$ cal./hr. This value is
approximately 0.5% of the total metabolic energy available.
But, if $B = U = 1.2$ moles/ l. (a 1.2 mole solution is iso-
tonic with 100% sea water), $W = 1.69$ cal./hr., and if $B = 0.320$ mole/l. with $U = M = 0.006$ mole/l., $W = 0.0187$ cal./hr.

Thus, as Eriocheir goes from sea water to fresh water, B
falls from 1.2 to 0.32 moles/l., and this reduced internal
concentration lowers the osmotic work from 1.69 to 0.0757
cal./hr. If this crab were able to produce a urine of
optimum concentration, (isotonic to the medium, $U = M$)
the osmotic work could be further lowered to 0.0187 cal./hr.
(Potts, 1954).

Potts makes the point that in fresh water, an animal
can reduce its osmotic work by as much as 90% by making its
urine isotonic with the medium, but even making it hypoton-
ic to the blood (while still several times more concentrated
than the medium) is extremely efficient. This is, in fact,
what most fresh-water animals do; none can produce a urine
isotonic with the medium. The reduction of the concentra-
tion of the urine is of maximum efficiency energy-wise in
the early phases of dilution. By lowering the urine con-
centration to 1/10 the concentration of the blood (ie. 5%
sea water), Potts calculates that 93% of the possible saving
on energy has been achieved, and the urine is still 100 times
more concentrated than the medium (.05% sea water).

Potts' hypothesis has been criticized because it is
based on the assumption of ideal semipermeability and
total solute loss via the urine. Shaw (1959) pointed out
that for many fresh-water Crustacea, most of the solute
loss occurs through simple diffusion across the body sur-

face. _Eriocheir_ loses only about 10% of its salt loss through the urine (Krogh, 1939). Furthermore, Shaw (1961b) states that most fresh-water Crustacea are not really semipermeable, and the saving in energy by the production of hyposmotic urine is not as great as Potts calculated. Lockwood (1961) noted that, although Potts' theory attributed little energy-saving advantage to hyposmotic urine in a brackish medium, _G. duebeni_ uses it in 50% or less sea water.

The comments above represent part of the disagreement concerning the order in which animals assumed the fresh-water adaptive mechanisms, and even concerning which mechanisms are basic and which are refinements. According to Beadle and Cragg (1940), the ability to produce a hypotonic urine was not the first step taken by animals in adapting to a fresh-water environment. Instead, they hypothesize, the power to actively transport salts from the medium into the blood came first, while the excretory organ still produced isotonic urine. _Carcinus_ can regulate in dilute sea water, but not in fresh water, and may be thought of as exemplifying this stage. _Eriocheir_ is quite similar but is found more often in fresh water because it has a somewhat better salt transport system. Parry (1954) noted that this two-stage hypothesis is acceptable only for animals with a very low permeability.

Animals without this would need a tremendous power of
ion absorption to offset solute loss. Ion exchange may
take place at the body surface, the gut, or the excretory
organ (Ramsay, 1954). Active transport of ions by surface
membranes is definitely a mechanism of hyper-osmotic reg-
ulation used by fresh-water animals, yet it probably ex-
isted in marine ancestors. <u>Cancer</u>, for example, keeps a
higher concentration of K than found in the normal medium,
yet possesses no osmoregulatory mechanisms.

Following the ability to actively transport salts
into the blood, according to the Beadle and Cragg hypothe-
sis, came the second stage in the transition to fresh water:
the ability to conserve salt by producing a hypotonic urine,
and the ability to reduce the strain of regulation by tol-
erating a lowering of the osmotic pressure of the blood to
about one-half the value found in marine animals. Since
the work of active transport depends upon the concentration
gradient to be overcome, the latter ability reduces reg-
ulatory work.

Shaw (1961a) disagrees with Potts about what is most
basic to fresh water adaptation. As mentioned above, Potts
(1954) considers lowered blood concentration and the pro-
duction of a dilute urine of greatest importance. Shaw
feels that these attributes are merely refinements, and
instead lists as most basic: 1) a lowered permeability and
2) a reduced external concentration at which saturation of

the high-affinity uptake mechanism is reached.

Active transport of salts was studied by Koch (1954) in isolated gills of Eriocheir. He found that they were capable of absorbing NaCl from a solution of only 1/30-1/40 the concentration of the blood. Absorption stops, however, in the absence of oxygen. Koch also showed that many basic dyes are reversible inhibitors of salt absorption, possibly because of anticholinesterase activity. Shaw (1961a) studied Na balance in Eriocheir and found that the mechanism for active uptake is saturated (working at top rate) at external concentrations as dilute as 6 mM/l.; this mechanism is adequate to balance the Na lost through the body surface. The oxidative activity of crab gill mitochondria has been studied as a function of the osmotic concentration of the medium by King (1966). She found that as the osmolarity of the medium decreased, the oxygen consumption of Callinctes sapidus gill mitochondria increased by 75% (for brackish-acclimated animals) and 35% (marine crabs). When the medium was diluted from 1.6 to 0.16 Osm., the specific activity of the gill mitochondria increased by 200-300%.

Bielawski (1964) did experiments with isolated podobranch gills of the crayfish Astacus leptodatylus Esch. and A. astacus L. The gills are permeable to water and usually allow a passive water intake, but Bielawski found

that gill permeability fell when the concentration of the
medium was lowered, so that regulation was facilitated.
Permeability rose as the concentration of the medium was
raised. Chloride and other ions are transported across
the gill membrane against a gradient. The carrier for Cl
is saturated at a medium concentration of 0.2%, and at
greater than 0.9% sea water the carrier is inhibited.
Shaw (1960) showed that in the crayfish Astacus pallipes
Lereboullet, Cl and Na transport is by two different
mechanisms which may act independently of each other.
Cl influx takes place in the presence of an electrochem-
ical gradient, and can occur at a high rate at lower
medium concentrations than does Na. A Cl deficiency rel-
ative to the Na concentration causes a change from Cl-Cl
exchange to an exchange of Cl for a useful anion, prob-
ably bicarbonate. Salt-depletion causes Na influx by
activating the Na transport system. Shaw's experiment
suggests that the Na system is the more basic to the main-
tenance of blood concentrations. After the Na level in
the blood is set, the relative concentration of Cl may then
activate the Cl system to bring the Cl level up to that of
Na.

The site of osmoregulation, if the area of transport
in the gills is in fact discrete, may have been detected
by Copeland (1963). The crab Callinectes is able to adapt

to fresh water from sea water. Copeland found near the afferent blood supply a patch of cells which he did not find in obligatory sea water forms, and believes that these may be the cells capable of hyposmotic regulation.

It is possible for animals to take in ions not only by transport mechanisms, but also through the food they eat. Parry (1961) studied Cl regulation in the Branchipod _Triops cancriformis_ Bosc and found that it could maintain a blood concentration well above the concentration of the medium. In its normal fresh water environment it is able to exclude the osmotic inflow of water while meeting salt requirements by food. In the laboratory it was able to live for six days in distilled water without food. The Entomostracans _Branchipus_ and _Chirocephalus_ also depend upon salt uptake from food. While _T. cancriformis_ maintains a blood concentration of 55 m.eq./l. in fresh water, by comparison, _Branchipus_ has a blood concentration of 30 m.eq./l. (Krogh, 1939), and _Chirocephalus_ (Panikkar, 1941) maintains 77 m.eq./l.

Triops longicaudatus, also a hyper-osmotic regulator, has been shown by Horne (1966) to depend more on an active uptake mechanism to maintain its necessary internal concentration of ions. Studied in pond water, _T. longicaudatus_ maintained a water to hemolymph ratio of 1:435 for Na, and 1:187 for Cl. This indicates a good regulatory mechanism.

In deionized water and with a food source, this animal lost both Na and Cl from the hemolymph, yet Ca and Mg concentrations were not significantly altered, and the concentration of K rose. _T. longicaudatus_, depending more on active uptake, is more permeable to ions than is _T. cancriformis_.

Brackish-water Crustacea

Brackish water is usually considered as being between 0.5‰ and 30‰ saline (Potts and Parry, 1964). It may be found in such places as estuaries, salt marshes, and inland seas. It is commonly believed that as crustaceans moved from the sea to fresh water, they often did so by first adapting to the transitional environment, brackish water. There is abundant evidence to support this theory. Sometimes species of the same genus are found which represent a spectrum of regulatory abilities. They may occupy niches which represent a spectrum of salinities, such as the decreasing salinity one may observe in going from the sea up a river.

The distribution of intertidal crabs in the Brisbane River was studied by Snelling (1959). The results of this study suggest that salinity is the most important factor controlling and limiting the range of estuarine crabs. Such crabs cannot extend into a given salinity unless they

possess the regulatory abilities necessary to maintain
their internal concentrations there.

Two populations of an estuarine isopod, Cyathura
polita Stimpson, were studied by Segal and Burbank (1963)
because of the salinity differences of their natural en-
vironments. One population was found in an estuary in
Massachusetts, with a tidal salinity variation of from
0.5‰ to 17‰, and an annual temperature fluctuation of
from about $2^{o}C$ to $23^{o}C$. The other population was found
in a spring in Florida, and lived in water of salinity
1‰, with probably less than $3^{o}C$ fluctuation in tempera-
ture annually. Both populations were able to hyper-osmo-
regulate in 50% to 3.5% sea water. When the medium con-
centration was increased experimentally to 75%-150% sea
water, the blood concentration was approximately isosmotic
with the medium. In water ranging from distilled to 3%
sea water, the estuary population was able to maintain
for a minimum of 48 hours the blood concentration it had
maintained at higher solution concentrations. The pop-
ulation from the spring, however, was unable to osmoreg-
ulate at salinities less than 3% sea water. At $32^{o}C$ the
estuary animals maintained about the same blood concen-
tration at varying salinities as they had at $22^{o}C$. The
osmoregulatory ability of the spring animals broke down in
either high or low concentrations at $32^{o}C$. Thus even

members of the same species may differ in their regulatory and adaptive powers. It is not difficult to believe that closely related animals may come to occupy separate niches due to different degrees of physiological adaptation to salinity differences.

Many estuarine animals can exist in water approaching the salinity of fresh water, indicating the ease with which a transition to a fresh-water environment might be made by some brackish-water forms. The Black Sea barnacle _Balanus improvisus_ occurs naturally in waters of salinity of 18‰. Turpaeva and Simkina (1961) found that it is able to withstand extreme dilutions: no harmful effects were observed at 5‰ but at 3‰ growth was slowed, and most died within a month in fresh water. The amphipod _Ampelisca spinipes_ Boeck was found in an estuarine environment (Nagabhushanam, 1965) and in experiments could tolerate salinities down to between 3‰ and .4‰.

It is necessary that animals maintain the osmotic pressure of their cells, whatever the salinity of the medium. Both marine and brackish animals use organic substances to maintain osmotic pressure. In fact, Lockwood (1962) states that inorganic ions contribute only about 1/3 to 1/2 of the osmotic activity in the marine Crustacea which have been studied. Lee and Mc Farland (1962) found that serum cations and Cl accounted for 95% of the osmotic pressure.

in the mantis shrimp <u>Squilla</u> <u>empusa</u> Say when the animal
was in normal sea water. As the medium became quite di-
luted, these ions contributed only 67% of the osmotic
pressure; the remainder was accounted for by an increase
in non-protein nitrogenous compounds. When the brackish-
water <u>Carcinus</u> <u>maenus</u> was transferred from 100 to 40% sea
water, Shaw (1958) found that organic substances were
contributing to the cellular osmotic pressure, since water
uptake and salt loss did not account for the isotonicity
of the blood and muscle fibers.

The basic mechanisms of ionic and osmotic regulation
in brackish-water Crustacea are like the mechanisms used
by fresh-water Crustacea, and the reader should refer to
the section dealing with fresh-water Crustacea for a
discussion and comparison. The mechanism of regulation
in the brackish-water prawn, <u>Metapenaeus</u> <u>monoceros</u>, will
be mentioned here because it is unlike the mechanisms
discussed previously. Gnanamathu (1966) studied this
prawn in a variety of medium concentrations, and found
that it increased in volume in a concentrated medium and
decreased in volume in a dilute medium. Fluid pressure
is involved in the mechanism of active regulation of water
across the gut wall. When the medium is hypertonic, water
is lost and the reduced hemolymph volume produces an in-
crease in pressure in the distended gut, causing an ultra-

filtrate of dilute sea water from the gut into the hemo-
lymph. The gut may alter the body volume, hence pressure,
by the influx or efflux of water. In a hypotonic medium,
the entry of water may put pressure on the gut and check
further entry of water, besides facilitating filtration of
hypotonic water from the hemolymph into the gut.

Marine Crustacea

The blood of most marine Crustacea, except prawns,
isopods, and grapsoid crabs, is isosmotic to sea water
(Lockwood, 1962), but almost nothing is known of osmo-
regulation in marine ostracods, copepods, cirripeds, mysids,
cumaceans, or euphasiids (Robertson, 1960). Those animals
which are in osmotic equilibrium still must use ionic
regulation because sea water does not have all ions in the
same proportions as they are needed in the body fluids of
marine animals. This regulation takes place by basically
two mechanisms: 1) selective excretion of ions by the
renal organs, and 2) controlled uptake of ions with water
from the medium. This uptake may be either by absorption
against a gradient, or perhaps by swallowing sea water and
excreting the excess salt across the gills, as is done
by teleosts. Gross (1955) showed that P. crassipes is
able to absorb water against a gradient.

In general, marine Crustacea produce a urine nearly

isosmotic with the blood. The antennal glands, then, are not concerned with osmoregulation but rather with controlling the ionic composition of the blood. Regulation usually involves maintaining the internal concentration of Na, K, and Ca above the concentration found in sea water, keeping the Mg and SO_4 concentrations lower than in sea water, and maintaining the Cl concentration close to equilibrium (Robertson, 1960). When the Mg and SO_4 concentrations are lower than found in sea water, the ionic concentration and cation-anion balance in the body fluids must be corrected. This is usually accomplished by raising the Na concentration and the Cl concentration, respectively.

A study by Dehnel and Carefoot (1965) dealt with blood and urine concentrations determined both in winter and summer for Hemigrapsus nudus and H. oregonensis. They detected no significant difference between winter and summer values for the blood ion concentration for Na over a range of 6% to 175% sea water; no difference for the K concentration over most ranges, except for being higher in the summer at a medium concentration of 100% to 150% sea water; a higher value for Ca in the winter than in the summer; and no significant difference for Mg. The summer urine Na concentration was approximately the summer blood Na concentration, indicating that an extra-renal mechanism is responsible for concentrating Na in the body. The

summer urine K concentration also was about equal to the blood K concentration, thus the antennary glands are shown to be ineffective for the regulation of K. In fact, regulation of ion concentration was shown to be extra-renal for all ions but Mg. The antennal gland is used to maintain a hypotonic* Mg concentration. This ability was impaired by increased temperatures.

Parry (1953) found that the isopod <u>Ligia oceanica</u> maintains an efficient ion regulatory system which keeps Na, K, Ca, and Cl concentrations in the blood hypertonic to sea water. The salts are probably derived from food or absorbed directly from the medium at some body surface such as the pleopods. The osmotic pressure of the blood can be regulated in salinities of from 20% to 100% sea water, but the blood concentration conforms to the medium concentration at values less than or greater than this, indicating a failure in the regulatory mechanism at these concentrations.

Research on ionic regulation in the palaemonid prawn <u>P</u>. <u>serratus</u> led Parry (1954) to hypothesize a regulatory mechanism involving an ultra-filtrate system. This prawn is normally hypotonic to sea water, with the blood concentration of Na, K, and Cl approximately 70-80% of the concentrations found in sea water. The Mg and SO_4 concentrations are lower in the blood than in sea water, and higher

*See note on page 11. This writer used tonicity if it was used by the author of the work cited.

in the urine than in the blood. The urine is isotonic
to the blood, and because the Na, K, Cl, and Ca concentra-
tions are about equal in the two solutions, Parry suggested
that the urine is an ultrafiltrate of the blood. Because
Mg and SO_4 concentrations were higher than in the blood,
she suggested that these ions were perhaps actively excret-
ed after formation of the ultrafiltrate. The antennal
gland of the palaemonid prawns does not have a site
such as the tubule of fresh-water crayfishes where salt-
absorption and osmoregulation take place. Parry states the
the end-sac of the antennal gland may be the site of the
ultrafiltrate production, and active excretion of Mg, SO_4,
and perhaps NH_3 may occur around the nephridial canal.
Reabsorption of ions to balance charges might take place
here also.

Riegal (1959) measured the changes in the total os-
motic concentration of body fluids in two sphaeromid iso-
pods, Gnorimi sphaeroma oregonensis Dana and Sphaer. pendoton
Richardson, after exposure for 3 to 48 hours in varying
salinities. In less than 50% sea water the animals were
hypertonic; from 75% to 125% sea water, they were usually
hypotonic. Because weight changes were not observed when
the internal concentration changed, the change took place
by salt movement. If the temperature was allowed to drop
from $16^\circ C$ to $5^\circ C$ there was a marked increase in weight

which suggests that under optimum temeratures the total
water content is actively maintained, and that low temp-
eratures must destroy the effectiveness of that mechan-
ism. The change in concentration observed at $5^{\circ}C$ could
not be explained only by the increase in water volume,
therefore salt movement was still taking place.

Terrestrial Crustacea

In a terrestrial habitat an animal is continually
losing water from its body by evaporation and in the
urine. Loss of water from body fluids means that the
internal concentration of ions will tend to rise, and may
in this way exceed in time the optimum concentration re-
quired for proper tissue functioning. Continued loss of
water may result in death. To ensure that the body fluids
do not become too concentrated, land forms have adopted
ways to reduce water loss. Means by which this may be
accomplished include the following:

1) Semi-terrestrial and terrestrial crustaceans have
an integument that is less permeable than is the integument
of marine forms (Edney, 1960). This condition prevents
great losses from the general body surface.

2) Land forms tend to reduce the surface area of
tissues used for gas exchange, eg. the gills (Nicol, 1960),
since so much evaporation takes place from the moist,

exposed tissues.

3) Terrestrial animals in general produce a rather small volume of urine.

Because these measures cannot eliminate all water loss, the animal may also seek to maintain its internal concentration by taking in water to dilute the ions which do become concentrated. To take up water, terrestrial crustaceans may eat moist food, drink from pools, enter water, or absorb water from sand. Gecarcinus lateralis is a brachyuran crab which lives a terrestrial life and is able to live indefinitely simply by exposure to sand dampened with fresh water (Gross, 1963). It is able to use the fresh water absorbed from the sand to dilute its body fluids, for it dies if sea water is the only source from which the sand is dampened. Flemister (1958) found that Gecarcinus, well removed from the marine life and living above the intertidal zones, was intolerant to either sea water or sand dampened with sea water.

Cardisoma carniflex, another brachyuran, can take up water against a gradient from sand dampened with either sea water or fresh water. The advantage to be gained in adaptation to terrestrialness may be questionable in this case, however, since the animal produces a urine hyposmotic to the body fluids after such rehydration, thereby losing water (Gross et. al., 1966). It is believed that terres-

trial forms evolved from marine forms, and Cardisoma
and Gecarcinus represent different degrees of evolution
away from a marine life. Cardisoma lives in the inter-
tidal and above, and will enter sea water and tolerate
high salinities. Gecarcinus lives away from the water,
and, as mentioned above, is intolerant to sea water.
Gecarcinus may even drown in water, which Cardisoma will
not do (Gross et. al. 1966).

Behavioral means of controlling water losses are
well-documented. Cardisoma guanhumi Latreille is very
independent of the water, but may enter sea water or
fresh water to take in water (Gifford, 1962). The ano-
muran land crab Coenobita cavipes was shown by Gross et.
al. (1966) to be able to control its water balance by
behavioral means. Pachygrapsus crassipes, which may be
considered to be a semi-terrestrial crab, has been shown
to prefer 100% sea water (Gross, 1957) to other salinities.
Because it can detect and avoid abnormal salinities, it
can sometimes prevent the osmoregulatory problems associated
with dilute or concentrated pools. This mechanism may
restrict its habitat to the intertidal and subtidal zones
rather than allow more freedom in range. Coenobita perlatus
will spend 90% of its time out of the water if allowed a
choice (Gross and Holland, 1960). It prefers to visit
sea water five times more often than fresh water, when it

does choose to enter water, possibly because its internal
concentration is not so high as to necessitate dilution
by fresh water. This assumption is supported by the fact
that, if its internal concentration is forced away from
the normal value, it seems to be able to select water having
a salinity which might best restore its internal concentra-
tion.

The role of food in affecting the internal concentra-
tion by contributing water was mentioned above. Food may
also add ions. Gross and Holland (1960) studied the ter-
restrial hermit crab Coenobita perlatus under a variety of
experimental conditions, including fresh water; fresh water
and food; fresh water and sea water; sea water and food;
fresh water, sea water, and food; and 150% sea water and
food. After 12 days in the experimental environments, the
crabs were analyzed for blood and urine concentrations.
When given fresh water and food, the blood Na, K, and Mg
remained fairly constant, regardless of whether or not sea
water was accessible. Given both sea water and fresh water,
an animal was able to maintain its internal concentration
without the aid of food. Given only fresh water, however,
the blood was less concentrated than when both food and
fresh water were available.

Because of the difficulties in precisely controlling
the internal concentrations, terrestrial forms may find

it advantageous to be able to tolerate wide fluctuations in the body fluid concentration. Coenobita perlatus can tolerate a Na concentration range of 83% to 220% of the normal value (Gross and Holland, 1960).

Terrestrial crustaceans are usually good hyposmotic regulators. Gross (1964) studied seven species of crabs, from a range of habitats, to determine their relative abilities to regulate. The crabs that he studied were the aquatic H. oregonensis and Cancer antennarius; the semi-terrestrial P. crassipes, Grapsus grapsus, Uca crenulata, and Ocypode ceratopthalma; and the terrestrial Gecarcinus lateralis. He found a definite correlation between the degree of terrestrialness of these crabs and their relative abilities as hyposmotic regulators.

The role of the antennal gland in ionic and osmotic regulation has been the subject of much research. Although both Cardisoma carnifex and Sesarmi meinerti were shown by Gross et al. (1966) to be strong hyper-osmotic and hyposmotic regulators, the antennal gland does not seem to be osmoregulatory in function, since the urine was isosmotic with the blood. It can help in ion regulation, however, by concentrating Mg in the urine independent of the Mg influx from the medium, and probably at the expense of Na. The ability to regulate Mg is not necessarily correlated with a low concentration of Mg in the blood, for Uca

crenulata was the best of the seven crabs studied by Gross
(1964) at concentrating Mg in the urine, yet it has a blood
concentration of Mg as high as does Cancer, with a poor
ability. Gecarcinus was found by Gross (1964) to be an
exception to the other terrestrial crabs in his study,
because it has only a poor ability to regulate Mg. He
hypothesized that Gecarcinus may have followed a different
evolutionary path of physiological adaptation to terrestri-
alness. If so, Coenobita perlatus has probably done so
also. In experiments with Coenobita perlatus, Gross and
Holland (1960) found that when the salinity of the external
medium was increased, the concentration of the blood Na
and Mg increased, K increased somewhat, and Ca did not
increase at all. They concluded that the antennary glands
are poor ion regulators. Coenobita may depend more on its
high tolerance for variation in its internal concentrations
than on ion regulation to live a terrestrial life. Gross
(1959) studied the ion-regulating powers of the antennal
gland of P. crassipes. It was ineffective in the regula-
tion of Na, K, and Ca, but much more effective in the reg-
ulation of Mg. Gross et al. (1966) presented evidence of
conservation of Ca by the antennal glands of C. carnifex
and S. meinerti by showing that the ratio of the urine Ca
to the blood Ca was less than 1. In general, the antennal
gland of terrestrial crustaceans is ineffective for osmo-

regulation, but can function in limited ion regulation,
most conspicuously for Mg.

Summary and Conclusions

Osmotic and ionic regulation in Crustacea has enabled
various members of the Crustacea to leave a marine envi-
ronment and to adapt successfully to brackish, fresh-water,
and terrestrial habitats. It has been shown that many
animals are limited in range basically due to a limited
ability to adapt to different salinities.

Fresh-water Crustacea generally have a low permeability
to water and salts. This condition lessens the dilution
of their body fluids by means of outward diffusion of salts
and water intake by osmosis. Ion absorption takes place
primarily across the gills by active transport. The energy
cost of this transport is proportional to the concentration
gradient existing between the internal body fluids and the
medium. By acquiring a toleration for less concentrated
internal fluids than their marine ancestors, fresh-water
forms reduce the energy cost of active transport. It is
to the advantage of fresh-water animals to have ion carriers
which saturate at very low external concentrations. Fresh-
water animals also benefit from producing a dilute urine.

Brackish-water crustaceans are more permeable than their
fresh-water relatives. The concentration of their body

fluids is in general greater than the concentration of
the medium. Existence in a brackish medium may be pos-
sible either because the animal is able to regulate its
internal concentration of ions or because it can toler-
ate large changes in the concentration of its body fluids.

Marine animals tend to be isosmotic with sea water.
There is thus little need of osmoregulation, but the need
for ionic regulation still exists. There is active ionic
uptake by the gills, and the antennal glands have a lim-
ited role in ion regulation. Some crustaceans are able
to actively maintain a hyposmoticity in sea water. The
means by which this is accomplished is deserving of more
research.

Terrestrial crustaceans must cope with a high rate of
water loss through evaporation and via the urine. It is
to their advantage to be quite impermeable. Water loss
is partially balanced by drinking, eating moist food, and
behavioral modes such as immersion in pools. Many ter-
restrial forms are able to regulate hyposmotically, while
others have adapted by toleration of wide fluctuations
in their internal concentrations.

The physiological and behavioral means to regulate os-
motically and ionically have been the subject of a great
deal of research. Although a large body of information
has accumulated dealing with decapods, there is a paucity

of information available on many of the other crustacean
groups. Research needs to be carried out on these forms
in order to obtain a more clear understanding of the pos-
sible variety of regulatory mechanisms. Active transport
is fundamental in any consideration of ionic or osmotic
regulation. The carriers involved in active transport are
not yet positively identified, and more research on the
histology and biochemistry of exchange membranes is needed.

Acknowledgement

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