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Optimal Composition of Chloride Cells for Osmoregulation in a Randomly Fluctuating Environment

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Abstract (187 words)

Fish live in water with a different osmotic pressure from that in the body. Their gills have chloride cells that transport ions to maintain an appropriate level of osmotic pressure in the body. The direction of ion transport is different between seawater and freshwater. There are two types of chloride cells that specialize in unidirectional transport and generalist cells that can switch their function quickly in response to environmental salinity. In species that experience salinity changes throughout life (euryhaline species), individuals may replace some chloride cells with cells of different types upon a sudden change in environmental salinity. In this paper, we develop a dynamic optimization model for the chloride cell composition of an individual living in an environment with randomly fluctuating salinity. The optimal solution is to minimize the sum of the workload of chloride cells in coping with the difference in osmotic pressure, the maintenance cost, and the temporal cost due to environmental change. The optimal fraction of generalist chloride cells increases with the frequency of salinity changes and the time needed for new cells to be fully functional but decreases with excess maintenance cost.

key words: euryhaline species, dynamic programming, ion transport, osmotic pressure.
Introduction

Teleost fish live in an environment whose osmotic pressure differs from that of the blood. To cope with the difference in osmotic pressure between the inside and outside of the body, they are equipped with chloride cells (or ionocytes) at their gills, which transport ions such as Na\(^+\) and Cl\(^-\) [1,2,3]. Fishes living in seawater have gill chloride cells specialized for living in seawater, in which the osmotic pressure is higher than that within the body. For example, the osmotic pressure in seawater is approximately 1,000 mOsm/kgH\(_2\)O and that in the body fluid of bony fishes is approximately 300 mOsm/kgH\(_2\)O [4]. Their chloride cells retain water and secrete liquid with a high ionic concentration. In contrast, fishes living in freshwater have gill chloride cells that function in the opposite manner because freshwater has lower osmotic pressure than the body.

Some fish species change their habitat in the course of their life, accompanied by changes in environmental salinity, exemplified by chum salmon and glass eel that migrate between freshwater and marine water. They are called euryhaline species [3]. Because the osmotic pressure of the blood is lower than that in seawater and higher than that in freshwater, chloride cells need to function in the opposite direction in the two environments [3,5,6]. The fish may replace their chloride cells at their gills. However, it takes some time for new chloride cells to become fully functional. Consequently, the change in the type of chloride cells causes the fish to cope with the osmotic pressure difference with fewer chloride cells, leading to a temporally increased workload for the osmoregulation system.

The delay in full functioning of chloride cells when the cells are replaced with new types is a very serious problem for species living in fluctuating salinity, such as fishes in tide pools or in estuaries and killifish, sea bass, and tilapia in brackish water.
To shorten the delay, there are chloride cells that can switch the direction of their function in a very short time (less than 24 hours) depending on the salinity in the environment [7,8,9]. These cells are equipped with ion pumps, transporters, and ion channels for both directions but use only those operating in a manner adapted to the current environment. We call these cells "generalist" chloride cells in the rest of this paper. Upon changes in environmental salinity, they switch their function quickly under the control of hormones, such as prolactin, cortisol, and growth hormone [10,11], and by the direct effect of the osmotic pressure of the medium [12].

In addition to generalist chloride cells that function in both directions, there are cells that specialize in osmoregulation in one direction. Some cells are adapted to function in seawater, and others are adapted to function in freshwater. We call these cells "specialized chloride cells". When the environmental salinity changes, the cell type composition may change, and new chloride cells of the type suitable to the new environment are formed from undifferentiated chloride cells, as shown by a study with Japanese sea bass [13]. This process takes some time because it involves cell differentiation. Another way to cope with changes in environmental salinity is to keep chloride cells that are unused until the environmental changes, when they become active immediately.

In this paper, we discuss the optimal composition of chloride cells for a fish living in an environment that switches between seawater and freshwater at random times. We consider a simple model in which there are three different chloride cell types: chloride cells specialized in living in seawater, chloride cells specialized in living in freshwater, and generalist chloride cells. We assume that the total number of chloride cells is a constant. If some cells are replaced by cells of different types, there is a period in which these newly formed chloride cells do not function fully. We search for the cell
composition that minimizes the workload of chloride cells to cope with the osmotic pressure difference between the inside and outside of the body plus the maintenance cost. We perform mathematical analysis based on dynamic programming and discuss how the composition depends on the frequency of environmental fluctuation, the maintenance cost difference between cell types, and the magnitude of time required for new chloride cells to become fully functional.

**Model**

Here, we consider the chloride cell composition of fishes living in brackish water with randomly fluctuating salinity. Let $x$, $y$, and $z$ be the numbers of saltwater-adapted chloride cells, freshwater-adapted chloride cells, and generalist chloride cells, respectively. The total number of chloride cells is a constant $T$. The set of all the cell compositions is:

\[ \Omega = \{(x, y, z) | x \geq 0, y \geq 0, z \geq 0, x + y + z = T; x, y, and \ z \ are \ integers\} \]

which consists of $T(T + 1)/2$ elements. Symbols $s$ and $f$ indicate living in saltwater and in freshwater, respectively.

We consider a period of a few months to half a year in which the focal fishes stay in brackish water. After this period, the fish may move to a different habitat, or it may proceed to a different life stage. We study the composition of chloride cell types and their response to environmental fluctuations, which minimizes the total workload of osmoregulation plus maintenance cost in this period. We assume that the length of this period follows an exponential distribution with expected length $1/\mu$, indicating that the individual leaves the habitat at a constant daily rate $\mu$.

**Salinity stress and maintenance cost**
Let $\theta$ be the osmotic pressure in the body and let $u_s$ and $u_f$ be the osmotic pressures in seawater and in freshwater, respectively. The osmotic pressure in the body is between $u_s$ and $u_f$ ($u_s > \theta > u_f$). The amount of salinity stress is proportional to the difference in osmotic pressure between the inside and outside of the body divided by the number of chloride cells that function to cope with the difference. It is

$$\frac{u_s-\theta}{x+z} \text{ in seawater}; \quad \frac{\theta-u_f}{y+z} \text{ in freshwater.} \quad (1)$$

In seawater, the osmotic pressure difference $(u_s - \theta)$ is addressed by seawater-adapted cells $x$ and generalist cells $z$, the latter working in both environments. In freshwater, the osmotic pressure difference $(\theta - u_f)$ is addressed by freshwater-adapted cells $y$ and generalist cells $z$.

To reduce the stress caused by the osmotic pressure difference between the inside and outside of the body, having only generalist chloride cells would seem to be beneficial. However, generalist chloride cells are more costly to maintain than specialist cells. Let $a_x$, $a_y$, and $a_z$ be the daily maintenance costs per cell for seawater-adapted cells, freshwater-adapted cells, and generalist chloride cells, respectively. We assume that $a_z > a_x$ and $a_z > a_y$. The total maintenance cost per day is $a_x x + a_y y + a_z z$.

**Temporally enhanced osmoregulation load due to chloride cell replacement**

Immediately after a change in the environmental salinity, some chloride cells may be replaced. Newly formed chloride cells become fully functional only after some maturation time. For example, if the environment changes from $s$ to $f$, the cell composition changes from $(x_s, y_s, z_s)$ to $(x_f, y_f, z_f)$. Some of the cells in $(x_f, y_f, z_f)$ cannot be functional for some period because they must be converted from the previous composition $(x_s, y_s, z_s)$. Let $\tau$ be the length of time needed for maturation. The cells of the old composition cope with the new salinity level (freshwater environment) less
well than \((x_f, y_f, z_f)\) in the period of length \(\tau\). This temporarily enhanced
osmoregulation load is an additional cost accompanying the change in chloride cell
composition.

To be concrete, the daily stress before the environmental change is
\((u_s - \theta)/(x_s + z_s)\), and the daily stress after the completion of changes in the chloride
cell composition is \((\theta - u_f)/(y_f + z_f)\). During a period of length \(\tau\) required for the
maturation of new chloride cells, the fish experiences daily stress with \((x_s, y_s, z_s)\) in a
freshwater environment. Hence, the daily stress is \((\theta - u_f)/(y_f + z_f)\), which is greater
than the daily stress after maturation \((\theta - u_f)/(y_f + z_f)\). The additional stress
accompanied by the change in cell types is:

\[
\tau(\theta - u_f) \left( \frac{1}{(y_f + z_f)} - \frac{1}{(y_s + z_s)} \right) \quad (2)
\]

We assume that the mean duration of each environment is much longer than \(\tau\).

**Dynamic optimization**

We consider the expected total future load if the current composition of
chloride cell types is \((x, y, z)\) and the current environment is \(i\), where \(i\) indicates
seawater \((i = s)\) or freshwater \((i = f)\). The individual chooses the chloride cell
composition immediately after a change in the environmental salinity. Consider a period
in which the fish is in seawater. Immediately before the period, the environment was
freshwater. The current period will end either when the next environmental change to
freshwater occurs or when the fish leaves the habitat, which occur at rates \(p\) and \(\mu\),
respectively. Hence, the length of the current seawater environment follows an
exponential distribution with mean \(1/(p + \mu)\). The expected future total load is

\[
\min_{x,y,z} \left[ \tau(u_s - \theta) \left( \frac{1}{(y' + z')} - \frac{1}{(x + z)} \right) + \frac{1}{p + \mu} \left[ u_s - \theta \left( \frac{a_x}{x + z} + a_y y + a_z z \right) \right] \right]
\]
\[
\begin{align*}
&+ \frac{p}{p+\mu} \min_{x',y',z'} \left\{ \tau (\theta - u_f) \left( \frac{1}{y+z} - \frac{1}{y'+z'} \right) + \frac{1}{q+\mu} \left[ \theta - u_f \right. \\
&\left. + a_x x'' + a_y y'' + a_z z'' \right] \\
&+ \frac{q}{q+\mu} \min \ldots \right\} \right\}
\end{align*}
\] (3)

where \((x, y, z)\) is the chloride cell composition in the current environment (seawater), \((x', y', z')\) is that in the previous environment (freshwater), and \((x'', y'', z'')\) is that in the next environment (freshwater). The minimization symbol indicates that the individual chooses the optimal chloride cell composition that achieves the smallest total future load. The choice must be made every time the environmental salinity changes. The first minimization symbol in equation (3) indicates the choice of \((x, y, z)\), which is made when the environment changes from freshwater to seawater, and the cell composition \((x, y, z)\) chosen is then adopted during the current period of a seawater environment, which lasts \(1/(p+\mu)\) days on average.

The first term after the square brace in equation (3) is the excess workload, which arises because the cell composition adopted before the environmental change (freshwater), denoted by \((x', y', z')\), is not well adapted to the new environment (seawater). The second term after the square brace is the expected duration of this environment \(1/(p+\mu)\) multiplied by the sum of the workload and maintenance cost per day.

The third term, written in the next line, is the total workload to be paid after the next environmental transition (from seawater to freshwater), multiplied by a factor \(p/(p+\mu)\), which is the probability that the environmental change stems from the end of the current period rather than the fish leaving the habitat. The terms in the curly brace after the second minimization symbol are mostly the decision to be made in the future. However, there is one term that is affected by \((x, y, z)\): the chloride cell composition in
the current seawater environment. This term arises because the fish must use the current

cell composition until newly formed cells become fully functional.

If we move some terms out of the minimization symbols, equation (3) can be
rewritten as

\[
\tau(u_s - \theta) \frac{x'}{x' + z'} + \min_{x, y, z} \left[-\tau(u_s - \theta) \frac{x}{x + z} + \frac{1}{p + \mu} \left[u_s - \theta + a_x x + a_y y + a_z z\right] + \frac{p \tau (\theta - u_s)}{p + \mu} + \min_{y', z'} \left[-\tau(u_s - \theta) \frac{y'}{y' + z'} + \frac{1}{q + \mu} \left[\theta - u_s + a_x x' + a_y y' + a_z z'\right] + \frac{q \tau (\theta - u_s)}{q + \mu} \right]\right]
\]

If we pay attention to the terms depending on the current choice of cell composition
\((x, y, z)\), we see that the optimal value can be calculated from the following:

\[
\min_{x, y, y, x, z} \left[(1 - (p + \mu)\tau) \frac{u_s - \theta}{x + z} + a_x x + a_y y + a_z z + \frac{p \tau (\theta - u_s)}{y + z}\right] \quad (4a)
\]

where minimization is calculated under \(x + y + z = T, \ x \geq 0, \ y \geq 0, \) and \(z \geq 0\).

This equation determines \((x_s, y_s, z_s)\), the optimal cell composition in the seawater
environment.

Similarly, we can calculate \((x_f, y_f, z_f)\) from the following:

\[
\min_{x, y, y, x, z} \left[(1 - (q + \mu)\tau) \frac{\theta - u_f}{y + z} + a_x x + a_y y + a_z z + \frac{q \tau (u_s - \theta)}{x + z}\right] \quad (4b)
\]

under \(x + y + z = T\).

Equations (4a) and (4b) are derived from assuming the excess workload
caused by the delay of new cell maturation is described by equation (2), which allows
us to move the term depending on the cell composition adopted in the current period
before the minimization symbol. We cannot perform this operation if the excess
workload has a more general functional form. Then, the optimal choice of chloride cell
composition cannot be obtained by the minimization of a single quantity, such as in
Equations (4a) and (4b). In such a case, we may have to adopt an analysis of iterative
calculations, which we explain in Appendix A of the Supporting Information.

By numerical analyses, we confirmed that these two methods (iterative and
noniterative calculation) give the same results. The method using equations (4a) and
(4b) is a much simpler and faster way to compute the optimal chloride cell composition
in each environment than the iterative calculation explained in Appendix A. In addition,
they sometimes provide simple and explicit mathematical expressions (see below).

**Optimal chloride cell composition**

Here, we discuss the dependence of the optimal chloride cell composition on
different parameters.

We first consider the situation in which the two environments are symmetric:
the environment switches between seawater and freshwater at an equal rate \( p = q \), the
maintenance costs are the same between the two specialist cell types \( a_x = a_y \), and the
magnitude of stress is the same although the direction is different \( u_s - \theta = \theta - u_f \).

Individuals contain one specialist cell type adapted to the current environment and the
other adapted to the next environment, although the current environment may be either
seawater or freshwater. Consequently, in the optimal solution, the number of generalist
cells is the same between the two salinity levels \( z_s = z_f \), but the numbers of seawater-
adapted specialist cells and freshwater-adapted specialist cells are switched between the
two situations \( x_s = y_f ; \text{ and } y_s = x_f \).

Figure 1 illustrates how the chloride cell composition changes at the
environmental shift in a symmetric situation. The horizontal axis indicates the time, and
the environment changes from seawater to freshwater at the time indicated by a triangle.

Figure 1a shows the case in which all the cells are generalists. No chloride cells change
their type. Figure 1b shows the case in which some chloride cells are generalists and others are specialists adapted to the current environment. When the environment changes, specialist cells adapted to seawater are replaced by specialist cells adapted to freshwater. Note that there is a time interval of length $\tau$ in which newly formed specialist cells are not functional (indicated by shaded area). Figure 1d illustrates the case in which no generalist cells exist, and two types of specialist cells occupy the chloride cell population, with the current functional type being more abundant than the other type. In seawater, specialist cells adapted to seawater are functional, but specialist cells adapted to freshwater are not functional. After environmental change, these cells become functional, while specialist cells adapted to seawater stop being functional. Some of them are replaced by specialists adapted to freshwater, which becomes functional only after time $\tau$ (indicated by shade). Figure 1c illustrates the case in which the chloride cell population includes all three cell types, in a mixture of Figs. 1b and 1d.

Four phases of chloride cell composition in seawater

The optimal chloride cell composition in seawater $(x_s, y_s, z_s)$ is determined from equation (4a). In this section, we illustrate how $(x_s, y_s, z_s)$ depends on various parameters. Derivations are explained in Appendix B.

Figure 2a illustrates the optimal composition of chloride cells in seawater. The horizontal axis indicates $a_z - a_x$, the excess maintenance cost of generalist chloride cells compared with that of the specialist chloride cells adapted to seawater. Depending on the magnitude of $a_z - a_x$, we have four distinct behaviors of the chloride cell population: Phases I, II, III, and IV, which are observed as the excess cost $a_z - a_x$ increases. We indicate these labels in Fig. 2a.
Phase I (All generalist cells)

When \( a_z - a_x \) is very small, all chloride cells are generalist cells in both environments.

\[ x_s = y_s = 0 \quad \text{and} \quad z_s = T. \]  

(5a)

This is the optimum when \( a_z - a_x < p\tau(\theta - u_f)/T^2 \). In this case, no cell replacement occurs at the time of environmental changes (see Fig. 1a).

Phase II (Generalists plus specialists adapted to the current salinity)

When \( a_z - a_x \) has an intermediate magnitude, the chloride cell population consists of generalist chloride cells and specialist chloride cells adapted to the current environment. Specialist chloride cells that are not adapted to the current environment are nonexistent. We can derive the optimal cell composition as follows.

\[
x_s = T - \frac{p\tau(\theta - u_f)}{a_z - a_x}, \quad y_s = 0, \quad \text{and} \quad z_s = \sqrt{p\tau(\theta - u_f)}.
\]

(5b)

In the case illustrated in Fig. 1b (situation is symmetric), generalist chloride cells can function in both directions, and they do not change cell type. In contrast, all specialist cells change from one adapted to the old environment to one adapted to the new environment.

Phase III (Mixture of all three cell types)

When \( a_z - a_x \) is large, all three chloride cell types are present. The solution is given as

\[
x_s = T - \sqrt{\frac{p\tau(\theta - u_f)}{a_z - a_x}}, \quad y_s = T - \frac{(1-(p+\mu)\tau)(u_s - \theta)}{a_z - a_y},
\]

and

\[
z_s = \sqrt{\frac{p\tau(\theta - u_f)}{a_z - a_x}} + \sqrt{\frac{(1-(p+\mu)\tau)(u_s - \theta)}{a_z - a_y}} - T.
\]

(5c)
The two specialist chloride cell types differ in number. There are more specialist cells that are adapted to the current salinity than the other type adapted to the future salinity ($x_s > y_s$). Note that the equation of $x_s$ is the same as that in Phase II. When environmental change occurs, some fraction of specialist cells switch to specialist cells of the other type (see Fig. 1c).

**Phase IV** (No generalist cells)

When $a_z - a_x$ is very large, no generalist cells are formed. Two types of specialist cells exist, but the one adapted to the current environment is more abundant than the other type of specialist (illustrated in Fig. 1d). In Appendix B of the Supplementary Information, we derived the optimal chloride cell composition in general cases.

For simplicity, we here show the explicit solution when $a_x = a_y$, indicating that the maintenance cost of the two specialized cell types is the same. Then, we have the following results. Because we assume that $\tau$ is much shorter than the length of each environment or the length of stay in the habitat, we can assume $(2p + \mu)\tau < 1$. If so, we have the optimal chloride cell composition as follows:

$$x_s = T \frac{\sqrt{\mu \tau}}{\sqrt{1-(p+\mu)\tau+\sqrt{\mu \tau}}}$$

$$y_s = T \frac{\sqrt{\mu \tau}}{\sqrt{1-(p+\mu)\tau+\sqrt{\mu \tau}}}$$

$$z_s = 0$$

(5d)

which is the case illustrated in Fig. 2a.

Figure 2b illustrates the optimal chloride cell composition in freshwater.

Because the situation is symmetric between two environments, $z$ is the same between Figs. 2a and 2b; $x$ in Fig. 2a is the same as $y$ in Fig. 2b; and $y$ in Fig. 2a is the same as $x$ in Fig. 2b.

**Parameter dependence**
The fraction of generalist chloride cells decreases as the environment changes less frequently, newly formed cells take a shorter time to be fully functional, and their excess cost increases. As shown in Fig. 2, generalist cells are replaced by specialist chloride cells, first by the one adapted to the current environment and then by the one not adapted to the current environment.

If we consider maintenance cost only, having specialist chloride cells adapted to the current environment would seem to be the most efficient. However, once the environment changes, these cells are not functional and must be replaced by new chloride cells of different types. These newly formed cells cannot function immediately; hence, the fish would suffer a large osmoregulation load during this period $\tau$. To cope with the risk of environmental change, the fish may have generalist chloride cells with a higher maintenance cost or may keep some specialized chloride cells that are not functional in the current environment but can function after the next environmental change. Whether to have generalist cells (Phase II), specialist cells that are not functional in the current environment (Phase IV) or both (Phase III) depends on the magnitude of excess maintenance cost of generalist cells $a_z - a_x (> 0)$, as shown in Figs. 2 and 3.

Figure 3 illustrates the dependence on $\tau$, the length of time required for newly formed chloride cells to be fully functional. All three parts of Fig. 3 illustrate the optimal cell composition in seawater. The overall dependence on $a_z - a_x$ is the same for different values of $\tau$. However, as $\tau$ increases, the number of specialized cells adapted to seawater $x$ decreases, and the number of generalist cells $z$ increases. In particular, the fraction of generalist chloride cells $z_s$ in Phase II increases, and the fraction of unused specialist cells $y_s$ in Phase IV increases.
The optimal chloride cell compositions in seawater and in freshwater are determined by equations (4a) and (4b), respectively. The optimal chloride cell population in seawater and that in freshwater may belong to different phases. There are three sources of asymmetry between the two environments and between the two kinds of specialized chloride cells.

(i) The transition rate between the two environments is different \((p \neq q)\)

Figure S1 in Appendix C of the Supplementary Information illustrates an example in which the rate of transition from freshwater to seawater is smaller than that of the reverse transition \((q < p)\). Hence, a larger fraction of the lifetime is spent in freshwater. Then, the high maintenance cost of generalist chloride cells would reduce the optimal value of \(z\) in freshwater. This is illustrated as the contrast between the two parts in Fig. S1.

(ii) Maintenance costs of the two specialist cell types differ \((a_x \neq a_y)\)

Figure S2 in Appendix C of the Supplementary Information illustrates an example in which the maintenance cost of specialist cells adapted to freshwater is larger \((a_x < a_y)\). This causes a smaller relative cost of generalist chloride cells in freshwater than in seawater. Hence, the optimal number of generalist chloride cells \(z\) is larger in freshwater than in seawater.

(iii) The workload of osmoregulation differs in the two environments \((u_s - \theta \neq \theta - u_f)\).

Figure 4 illustrates the dependence of chloride cell composition on \(\theta\), the osmotic pressure in the body. The three parts indicate different costs of generalist cells
\( \alpha_z \): (a) \( \alpha_z = 0.05 \); (b) \( \alpha_z = 0.1 \); and (c) \( \alpha_z = 0.2 \). As \( \theta \) increases, the number of specialist chloride cells adapted to seawater \( x \) decreases and the number of generalist chloride cells \( z \) increases. There exists a peak value of \( z \) at an intermediate value of \( \theta \). After this peak, the number of generalist cells \( z \) decreases and the number of specialist cells adapted to freshwater \( y \) increases with \( \theta \). When \( z \) approaches zero, \( y \) increases and \( x \) decreases with \( \theta \). As \( \theta \) increases, the chloride cell composition changes from Phase II (\( z > 0, \ y > 0 \)) to Phase III and then to Phase IV. If we compare three parts with different costs of generalist cells \( \alpha_z \), the fraction of generalist cells decreases.

If \( \theta \) is small and close to \( u_f \), the workload for osmoregulation is smaller in freshwater than in seawater. The opposite is the case when \( \theta \) is large and close to \( u_s \). In both extreme cases, the optimal cell composition is dominated by specialized cells adapted to the environment with a heavier workload. The optimal number of generalist chloride cells \( z \) has a peak at an intermediate value of \( \theta \).

In Fig. S3 in Appendix C of the Supplementary Information, we explained a similar figure with three different values of \( \tau \). As \( \tau \) increases, the optimal number of generalist chloride cells increases.

**Discussion**

Upon a change in environmental salinity, various tissues, including gill, kidney, and intestine, exhibit changes in their osmoregulatory functions [14]. Among these, the response of tissues in the gills is very important and has been the subject of intensive physiological and morphological studies [2,15]. Fishes living in brackish water, such as killifish, sea bass, and tilapia, have gill chloride cells of several different types [8,16]. Chloride cells of one of these types include molecular machinery.
functioning in osmoregulation in both directions, and they activate the function that is
adaptive and suppress the function that is not adaptive in the current environment. Upon
environmental change, they quickly change their function (less than 24 hours) [7,9]. We
called them generalist chloride cells in this paper. In addition, newly developed chloride
cells are formed from undifferentiated chloride cells [13]. We expect the latter process
to require a longer time to complete.

In this paper, we discussed the optimal composition of chloride cells in a
randomly fluctuating environment. If the cost in the current environment were the only
concern, the optimal strategy would be to have specialist cells that are adaptive in the
current environment, similar to species living only in seawater or only in freshwater
(stenohaline species). However, this strategy is quite risky if there is a chance for the
environmental salinity to change.

We distinguished two forms of adaptive responses to a sudden salinity
change: (i) generalist chloride cells change the direction of their function immediately,
and (ii) some cells may be replaced by chloride cells of different types. The latter
response should require a longer time to complete than the former because it includes
the process of cell development from undifferentiated cells. Having generalist chloride
cells is more costly in maintenance than specialist cells, and the cell type change causes
a period in which functional chloride cells in the new environment are fewer than in the
stationary environment.

We formalized a model of the optimal composition of chloride cells and
analyzed it by dynamic programming. The optimal solution is one of the four
qualitatively different mixtures of chloride cell types, Phases I, II, III, and IV,
depending on the excess cost of generalist cells, frequency of environmental changes,
time required for new chloride cells to become functional, and workload of
osmoregulation. When the excess cost of generalist cells is very small, all chloride cells are generalists that change to the adaptive function almost immediately after the environment changes (Phase I, see Fig. 1a). When the excess cost of generalist cells is only slightly larger, the optimal composition includes both generalist cells and specialist cells of a type adaptive to the current environment (Phase II, see Fig. 1b). These specialist cells may change their type when the salinity suddenly changes. In contrast, if the cost of generalist cells is very large, the optimal composition includes no generalist cells but always includes specialist cells of both types (Phase IV, see Fig. 1d). The fraction of specialist cells that are adaptive in the current environment is larger than the fraction of specialist cells of the other type, but the latter become active once the environment changes. Between Phase II and Phase IV, there are situations in which the optimal cell composition includes chloride cells of all three types (Phase III, see Fig. 1c).

To cope with the possibility of environmental change, the individual must pay some cost. Having generalist chloride cells is more costly than having specialist cells. Keeping specialized cells that are not functional in the current environment would also be costly because this decreases the number of functional chloride cells in the current environment and increases the workload of osmoregulation.

In general, we have a situation in which two environments are asymmetric because the rates of environmental change, the maintenance costs of specialist cells, and/or the workload of osmoregulation differ between the two environments. Then, the optimal chloride cell composition in each environment may belong to the same or different phases, as shown in Fig. 4 and in several figures in Appendix C of the Supporting Information.
A key assumption of the model studied in the present paper is that the cost of
the replacement of cells by cells of different types is caused by the delay before new
chloride cells to become fully functional. A similar effect of delay in phenotypic
transition was studied for sex change in coral reef fishes [17]. In most species of sex-
changing fishes (for example, from female to male), sex change requires time for the
development of the gonad of the new sex (testis), which is from several weeks to
several months [18]. Some sex changers keep the gonads of both sexes all the time, but
the gonad of one sex stays inactive, as exemplified by Trimma okinawae [19]. When the
social situation changes, the individual changes the sex within a relatively short time
(one or two weeks). On the other hand, keeping the currently unused gonad could incur
costs. Dynamic programming analysis showed that the advantage of keeping gonads
that are not currently in use depends on the frequency of social status changes [17].

The length of time required for the development of new chloride cells $\tau$
plays a very important role in the current model for gill adaptation to changes in
environmental salinity. Evaluating $\tau$ quantitatively is an important theme of future
study.

In this paper, we discussed a model incorporating unpredictable changes in
salinity, which is suitable for fishes living in brackish water. In contrast, in some
species, the transition from one habitat to another with different salinities is a part of the
normal life cycle [20]. For example, catadromous fishes, such as eels, have breeding
sites in the sea, swim upstream to grow and then go down to the ocean when they
mature, while anadromous fishes, such as salmon, have spawning sites in the stream and
growing habitats in the ocean. The timing for the change in salinity should be
predictable for each individual, and the fish may be able to change chloride cells before
the transition to a habitat with different salinity.
Suppose, for example, that the salinity change from freshwater to seawater can be predicted beforehand. Fishes may have all freshwater-adapted chloride cells much before the transition, and as the transition time (e.g., the time of going down to the ocean) is approaching, they may replace those specialist cells with generalist chloride cells. After they went down the ocean, generalist chloride cells may be replaced again by specialist chloride cells adapted in seawater. Calculating the optimal schedule of having different chloride cells given such a predictable change in environmental salinity and comparing the model with the case studied in this paper would be an important theme of future theoretical study.

The model studied in this paper includes many simplifying assumptions. First, we assumed a single type of specialized chloride cell adapted to the freshwater environment, but multiple types of chloride cells are adapted to the freshwater environment [21,22]. Second, although we assumed that the number of chloride cells was a fixed constant, the number of mature chloride cells might fluctuate over time. The spatial distribution of chloride cells changes upon an environmental change [13,16]. Third, the generalist cell response to environmental changes depends both on hormones and on the direct change in osmotic pressure experienced by the focal cells, suggesting that hormones might coordinate different cells and tissues [10,11,12]. Although only the gills of fish were discussed in this paper, tissues in the intestine and kidney are known to respond to the change [14], which might affect each other by hormones.

Notwithstanding these many simplifications, the model is worth examining because it is the first attempt to theoretically analyze the phenomena of the osmoregulation of fish gills. It demonstrates how the cost, the time required for cell maturation, and the likelihood of future environmental changes would produce different responses of organisms. The chloride cells in the gills of euryhaline fishes should be one
of the best studied systems for adaptation and evolution in fluctuating environments [3, 23, 24].
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Author Contributions

Y.U., Y.I. and S.Y. designed the study, performed the study, and wrote the paper.

Additional Information

Competing Interests

The authors declare no conflict of interest.

Data Availability

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Supplementary Information

See an attached file (SI_Appendices).
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**Figure legends**

**Figure 1** Scheme illustrating the effect of the time required for new chloride cell maturation. Horizontal axis is for time. Symmetric situations are used for illustration. The environment changes from seawater to freshwater at the point indicated by the triangle. (a) All chloride cells are generalists (Phase I). (b) A mixture of generalist cells and one type of specialist (Phase II). (c) A mixture of all three types (Phase III) and two types of specialist cells but no generalists (Phase IV).

**Figure 2** Optimal number of chloride cells of three types. (a) In seawater. The horizontal axis is $a_z - a_x$, the excess maintenance cost for the generalist cell. (b) In freshwater. Horizontal axis is $a_z - a_y$. Four cases are indicated by Roman numbers (I, II, III, IV) (see text for explanation). Broken curves and lines are for the prediction of simple formulas. The parameters are $p = 0.05$, $q = 0.05$, $\mu = 0.01$, $u_s = 1000$, $u_f = 0$, $\theta = 500$, $a_x = 0.01$, $a_y = 0.01$, $\tau = 2$, and $T = 50$.

**Figure 3** Optimal numbers of chloride cells for different $\tau$. Here, we show the composition in saltwater only. Three parts are the results for different $\tau$, the length of time needed for newly formed cells to function: (a) $\tau = 0.5$, (b) $\tau = 2.0$, and (c) $\tau = 5.0$. As $\tau$ increases, $z_s$ increases in Phase II, and $y_s$ increases but $x_s$ decreases in Phase IV. Other parameters are $p = 0.05$, $q = 0.05$, $\mu = 0.01$, $a_x = 0.01$, $a_y = 0.01$, $u_s = 1000$, $u_f = 0$, $\theta = 500$, and $T = 50$.

**Figure 4** Optimal number of chloride cells of three types in seawater. Horizontal axis is $\theta$. Three parts have different excess maintenance costs for generalist cells. (a) $a_x = 0.05$; (b) $a_x = 0.1$; and (c) $a_x = 0.2$. Horizontal axis is $\theta$, osmotic pressure of the 
body. Three cases are indicated by Roman numbers (II, III, IV) (see text for explanation). The number of generalist chloride cells has a peak in the middle value of $\theta$. Other parameters are $\mu = 0.01$, $p = 0.05$, $q = 0.05$, $a_x = 0.01$, $a_y = 0.01$, $u_s = 1000$, $u_f = 0$, $\tau = 2$, and $T = 50$. 


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Figure 1

See image above for figure legend
Figure 2 Optimal number of chloride cells of three types. (a) In seawater. The horizontal axis is \( z - x \), the excess maintenance cost for the generalist cell. (b) In freshwater. Horizontal axis is \( a_z - a_y \). Four cases are indicated by Roman numbers (I, II, III, IV) (see text for explanation). Broken curves and lines are for the prediction of simple formulas. The parameters are \( p = 0.05 \), \( q = 0.05 \), \( \mu = 0.01 \), \( n_x = 1000 \), \( n_y = 0 \), \( \theta = 500 \), \( a_x = 0.01 \), \( a_y = 0.01 \), \( \tau = 2 \), and \( T = 50 \).

Figure 2

See image above for figure legend
Figure 3  Optimal numbers of chloride cells for different $\tau$. Here, we show the composition in saltwater only. Three parts are the results for different $\tau$, the length of time needed for newly formed cells to function: (a) $\tau = 0.5$, (b) $\tau = 2.0$, and (c) $\tau = 5.0$. As $\tau$ increases, $z_\tau$ increases in Phase II, and $y_\tau$ increases but $x_\tau$ decreases in Phase IV. Other parameters are $p = 0.05$, $q = 0.05$, $\mu = 0.01$, $a_x = 0.01$, $a_y = 0.01$, $u_y = 1000$, $u_f = 0$, $\theta = 500$, and $T = 50$.

Figure 3

See image above for figure legend
Figure 4 Optimal number of chloride cells of three types in seawater. Horizontal axis is $\theta$. Three parts have different excess maintenance costs for generalist cells. (a) $\alpha_x = 0.05$; (b) $\alpha_x = 0.1$; and (c) $\alpha_x = 0.2$. Horizontal axis is $\theta$, osmotic pressure of the body. Three cases are indicated by Roman numbers (II, III, IV) (see text for explanation). The number of generalist chloride cells has a peak in the middle value of $\theta$. Other parameters are $\mu = 0.01$, $p = 0.05$, $q = 0.05$, $\alpha_y = 0.01$, $\alpha_z = 0.01$, $u_z = 1000$, $u_f = 0$, $r = 2$, and $T = 50$.

See image above for figure legend

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