

REVIEW

# Overview of trace element trophic transfer in fish through the concept of assimilation efficiency

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**ABSTRACT:** Fish bioaccumulate trace elements both from the surrounding water (across the gills) and through diet (via the gastrointestinal tract), with diet generally being the major contributor. A laboratory-based approach is currently the most appropriate way to precisely quantify the trophic transfer of trace elements in fish, and assimilation efficiency (AE) of trace elements from ingested food is a commonly determined parameter. However, there are still some discrepancies in the literature regarding the definition and the determination of AE in aquatic organisms and especially in fish. In this paper, we review the literature to provide a consolidated definition of the concept of AE as well as a description of the methods and protocols used to quantify the AE of trace elements. We also review the main studies of trace element AE in fish. Most studies reporting AE considered the effects of biotic factors, especially the influence of the quality of food, whereas abiotic factors have received less attention, although they affect fish physiology and, by extension, potentially affect the AE of trace elements. The need for further studies is thus noted, especially the influence of abiotic factors such as temperature, salinity or pH on trace element AE or in the context of the co-occurrence of multiple stressors; this will help us to better understand the trophic transfer of trace elements and thus their overall bioaccumulation in fish.

**KEY WORDS:** Assimilation efficiency · Fish · Food · Metals · Experimental studies · Review

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## INTRODUCTION

In the field of ecotoxicology, scientific studies on fish originated in the 1930s (e.g. Jones 1938, 1939) with the purpose of testing the effects of various chemicals on fish, including toxic trace elements usually released in aquatic environments by anthropogenic activities (Förstner & Wittmann 2012). Since then, fish have proved their suitability for ecotoxicological studies (Braunbeck et al. 1998), given their broad species diversity, the wide range of diets (from

algae to other fish) and their broad geographical distribution in various environments. Furthermore, the relevance of fish in ecotoxicology is also connected to their ecological and economic importance as well as to the fact that they are important components of environmental risk assessments (Holmlund & Hammer 1999, Tidwell & Allan 2001).

Fish accumulate trace elements through both the dissolved and particulate pathways, but diet appears to be the predominant source for many elements (e.g. Xu & Wang 2002, Mathews & Fisher 2009). There-

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fore, understanding the trophic transfer of trace elements is a key aspect in assessing accumulation capacities in fish and their exposure to contaminants. Since distinguishing the contribution of diet to overall bioaccumulation is complex to perform on individuals collected in the field, the experimental approach appears to be the best option to assess unambiguously the trophic transfer of trace elements in fish (Wang & Fisher 1999).

One of the most relevant parameters for quantifying trophic transfer of a contaminant is the assimilation efficiency (AE) from ingested food. AE is a first-order physiological parameter that can be compared quantitatively among trace elements, fish species, diets and environmental conditions (Wang & Fisher 1996, Croteau et al. 2007). Because dietary trace element bioaccumulation is directly related to AE, this parameter is important in order to understand and predict global trace element uptake (Wang & Fisher 1996, Luoma & Rainbow 2005, Croteau et al. 2007). It is thus widely used in modern ecotoxicology studies. However, the concept of AE sometimes appears unclear in the literature due to some discordances in the way it is defined.

This review provides a general definition of the concept of AE, critically examines the methodologies used to date for AE measurements in fish and discusses the recent improvements made on the different methods. It also extensively analyses the results of trace element AEs in fish reported in the literature. Finally, it presents a summary of perspectives for guiding future studies on the subject. The review complements the one on AE in invertebrates by Wang & Fisher (1999).

#### THE NEED TO CLEARLY DEFINE THE CONCEPT OF AE

AE is a physiological parameter determined to understand the trophic transfer of chemicals in organisms. However, as Wang & Fisher (1999) pointed out in their review, there are still discrepancies in experimental studies regarding the definition of AE. According to those authors, 'In bioenergetic studies, absorption of an element or compound equals total ingestion of the substance minus its quantity in faecal matter and is the sum of assimilation and post-digestive soluble excretion (i.e., loss of material into the dissolved phase after post-ingestive metabolism)' (p. 2034). According to this definition, AE is the fraction of the ingested element or compound that is incorporated into biological tissue,

whereas absorption efficiency is the fraction of the ingested element or compound that passes through the gut epithelium by passive and active transports (Brett & Groves 1979, Penry 1998). Assimilation thus equals absorption minus defecation and excretion. This definition of AE is in line with Warnau et al. (1996), who indicate that AE could be defined as the fraction of the ingested material that is tightly bound (i.e. incorporated) in the organs and tissues of a given organism. From a theoretical point of view, the difference between absorption and assimilation is obvious, but in practice, it is difficult to delineate quantitatively these 2 mechanisms at the whole-body level, because during gut transit, these physiological processes can occur at the same time. Thus, another physiological parameter is used to determine the required time to assess AE (e.g. Ni et al. 2000, Xu & Wang 2002): gut transit time (GTT), i.e. the duration that a food ration spends in the digestive tract between its ingestion and its defecation. It is during this phase that the absorption of chemicals takes place. AE measurement based on GTT determination has some limitations, which must be taken into account. During GTT, it is difficult to ensure that only absorption of the ingested compounds takes place, since excretion can also already intervene; hence a part of the absorbed fraction can already have been excreted. After intestinal absorption, compounds or trace elements are conveyed through the bloodstream first to the liver and are then distributed to various organs via the heart. However, a part of them can be directly excreted via biliary secretions discharged into the intestine, or later through the gills and the urine (Wood 2011). Furthermore, there are some assumptions that egestion directly from the gut can occur through compounds secreted with digestive juices or sloughed inside detached enterocytes and then evacuated via the faeces or rectal fluid (Wood 2011). In addition, we assume that part of the non-assimilated fraction might remain somewhat longer in the digestive tract, associated to the intestinal mucus, which can play a regulatory role in the absorption of ingested elements such as trace elements (Warnau et al. 1996, Bury et al. 2003). These factors may thus affect the accuracy of AE determinations. This fact raises the crucial importance of the design and duration of experiments (i.e. the duration of the feeding period and the time during which depuration is followed after ingestion of food) in order to accurately determine AE (see also 'How the duration of depuration influences AE determination' below).

## DETERMINATION OF AE IN FISH

### AE of macromolecules in fish

Sometimes AE of a given element or compound is calculated as the difference between quantity ingested (quantity present in the food) and quantity egested (quantity in the faeces). This method, the so-called mass-balance, has been used to study the AE of nutrients such as proteins and lipids in farmed fish. Using this method, AE can be calculated as follows:

$$AE (\%) = \left( \frac{\text{ingested} - \text{faecal}}{\text{ingested}} \right) \times 100 \quad (1)$$

However, urinary and branchial excretions are not taken into account in this calculation, which limits its accuracy. Furthermore, to be efficient, the mass-balance approach requires an accurate quantification of the studied compound in the food and the faeces. Challenges may appear at this stage, such as the ability to collect faeces before their complete or partial dissolution in the water, which could lead to the loss or partial loss of the studied element (Choubert 1999).

Another method, based on the same mass-balance principle, uses an inert tracer, such as  $\text{Cr}_2\text{O}_3$  (Austreng 1978, Austreng et al. 2000),  $\text{TiO}_2$  (Weatherup & McCracken 1998, Vandenberg & De La Noüe 2001, Richter et al. 2003) or acid-insoluble ash (Sarker et al. 2016). Incorporated in the compounded feed or ingredients/constituents of the food matrix (Tacon & Rodrigues 1984, Morales et al. 1999), the inert tracer allows correcting the AE measurement for possible post-egestion loss. In this case, AE can be calculated using the following equation (Maynard & Loosli 1969):

$$AE (\%) = \left( 1 - \frac{\% \text{ inert marker in the food}}{\% \text{ inert marker in the faeces}} \times \frac{\% \text{ element in the faeces}}{\% \text{ element in the food}} \right) \times 100 \quad (2)$$

This ratio is widely used in aquaculture nutrition since it does not require a complete recovery of faeces, as is the case for the original approach. Its use is nevertheless limited nowadays given the fact that the selected inert marker must fulfil several characteristics, which are not easily met. The inert marker, in principle, should: (1) be absolutely inert, without a physiological effect on the fish; (2) not be absorbed or metabolized; (3) not influence absorption and/or digestion; and (4) be easily and quickly measurable (Choubert 1999). To the best of our knowledge, no marker perfectly fits all these conditions at once. Furthermore, this method does not take into account urinary and branchial excretion. Despite some dis-

advantages, this method is however still used in aquaculture studies to determine the AE of macromolecules such as proteins and lipids in fish (e.g. Sarker et al. 2016). With an increasing research interest in the trophic transfer of trace elements in fish, other methods for AE determination, developed specifically for these elements, have emerged.

### AE of trace elements in fish

#### Use of radiotracers

One of the most efficient methods to determine AE of trace elements in fish is the use of radiotracers. As radioisotopes have similar biochemical properties to their non-radioactive analogous isotopes, they can be used as tracers to follow an element in an organism. Thus, the 2 approaches described in the previous section (mass-balance and ratio; see 'AE of macromolecules by fish' above) can be applied in the determination of AE for trace elements, using radiotracers in aquatic organisms such as fish.

In addition to the 2 previous methods, the use of radiotracers and particularly gamma-emitting radiotracers has allowed the development of an efficient approach in the determination of the AE of trace elements: the pulse-chase feeding method. It has many advantages that explain its widespread use in the literature (e.g. Xu & Wang 2002, Wang et al. 2012, Pouil et al. 2016). The use of gamma-emitting radioisotopes allows radio-counting fish alive, thus limiting the number of individuals to sacrifice and generating data with reduced biological variability (Warnau & Bustamante 2007). In the pulse-chase feeding method, fish are fed radiolabelled food (natural prey or compounded feeds) and are radio-counted just after the radiolabelled feeding. Then, fish are regularly counted alive in order to describe the depuration kinetics of the radiotracers and thereby to determine the AE (see details below in 'How the duration of depuration influences AE determination'). The determination of AE based on a kinetic approach is done from a single feeding with a radiolabelled food item. The fish are allowed to feed on the radiolabelled food for a short period of time (shorter than their GTT; usually from 5 min to 2 h) to ensure that the radioactivity ingested can be accurately quantified without any possible radiotracer recycling from seawater due to leaching from the radiolabelled food, leading to an overestimation of AE. Recently, Pouil et al. (2017b) provided an experimental validation of the single-feeding method for the determination of Co, Cd, Mn

and Zn AEs in turbot *Scophthalmus maximus* fed with radiolabelled compounded food. Ni et al. (2000) had earlier compared AEs of Cd, Cr and Zn in the mudskipper *Periophthalmus modestus* and the glassy *Ambassis urotaenia* obtained using mass-balance and kinetic approaches, and concluded that the 2 approaches give similar results.

### Improvements in the AE calculation

Two methods are commonly used to calculate trace element AE using gamma-emitting radiotracers. For both methods, the proportion of trace elements retained in the fish during the depuration period is followed using regular gamma counting of live organisms. In the first method, AE is determined at a given time and expressed as a percentage of trace element retained after the GTT from the total ingested fraction (e.g. Xu & Wang 2002, Van Campenhout et al. 2007, Goto & Wallace 2009). Usually, in this method (the 'short-term' approach), the depuration is followed over a short time (i.e. a few hours or a few days; Table 1); it therefore provides a rapid insight into the transfer of trace elements in fish from their food. The second method is based on the actual determination of the trace element depuration kinetics. This method has been extensively used in radioecological studies on aquatic organisms and is improved by the use of multi-exponential models, in which parameters are solved by iterative adjustment (e.g. Warnau et al. 1996, Bustamante et al. 2002, Metian et al. 2010, Pouil et al. 2016). Depuration of trace elements is typically expressed as the percentage of remaining radioactivity (radioactivity at time  $t$  divided by the initial radioactivity measured in the organism at the beginning of the depuration period  $\times 100$ ). Depuration kinetics are generally best fitted by a 2-component exponential model:

$$A_t = A_{0s} \times e^{-k_{es}t} + A_{0l} \times e^{-k_{el}t} \quad (3)$$

where  $A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively;  $k_e$  is the depuration rate constant ( $d^{-1}$ ). The 's' and 'l' subscripts are related to the short- and long-lived component, respectively. The 's' component mainly represents the depuration of the radiotracer fraction that is weakly associated with the organisms and rapidly eliminated (i.e. the radiotracer fraction associated with the faeces). The 'l' component mainly describes the depuration of the radiotracer fraction that is actually absorbed by the organism and eliminated slowly (Hubbell et al. 1965, Reichle 1967, Reichle et al. 1970, Whicker & Schultz 1982,

Table 1. Assimilation efficiencies (AE) of trace elements and methylmercury (MeHg) (designated in the table as metals) in fish reported in experimental studies

| Fish species                            | Study objectives   | Metal | Food                                 | Duration of depuration (d) | AE (%)       | Reference  |
|---|--|-------|--------------------------------------|----------------------------|--------------|--|
| <i>Acanthopagrus schlegelii</i>         | Allometry, Feeding ratio and frequency, Food composition, Trace element pre-exposure | Ag    | Crustaceans                          | 2                          | 10–41        | Dang et al. (2009), Guo et al. (2015), Long & Wang (2005b), Wang et al. (2012), Zhang & Wang (2005), Zhang & Wang (2007) |
|   |  | Cd    | Crustaceans, fish, molluscs, pellets | 1.5–2                      | 2–38         |  |
|   | Hg(II)<br>Zn   | Cu    | Crustaceans, molluscs, pellets       | 2–2.5                      | 2–11         |  |
|   |  | Zn    | Pellet<br>Crustaceans, pellets       | 2<br>1.5–2                 | 3–55<br>2–50 |  |
| <i>Ambassis urotaenia</i>               | Interspecific comparison, Food composition   | Cd    | Crustaceans                          | 1                          | 10–43        | Ni et al. (2000)   |
|   |  | Cr    | Crustaceans                          | 1                          | 1–10         |  |
|   |  | Zn    | Crustaceans                          | 1–2.1                      | 2–32         |  |
| <i>Amphiprion ocellaris</i>             | Water pH   | Mn    | Pellets                              | 20                         | 1–10         | Jacob et al. (2017)  |
|   |  | Zn    | Pellets                              | 20                         | 24–35        |  |
| <i>Cyprinodon variegatus variegatus</i> | Pharmacokinetic model  | MeHg  | Phytoplankton, pellets               | 0.1–35                     | 38–100       | Leaner & Mason (2002)  |
| <i>Cyprinus carpio</i>                  | Food composition and quantity, Water temperature                                     | Cd    | Insects, oligochaetes, molluscs      | 2                          | 9–80         | Van Campenhout et al. (2007)   |
|   |  | Zn    | Insects, oligochaetes, molluscs      | 2                          | 20–97        |  |

(Table continued on next page)

Table 1 (continued)

| Fish species                     | Study objectives                             | Metal                                     | Food  | Duration of depuration (d) | AE (%)      | Reference                                     |       |                       |  |
|----------------------------------|--|---|---|----------------------------|-------------|---|-------|-----------------------|--|
| <i>Danio rerio</i>               | Food composition, Trace element pre-exposure | Ag  | Polychaetes                                       | 3                          | 1-7         | Boyle et al. (2011), Liu et al. (2002)        |       |                       |  |
|                                  |  | Cd  | Crustaceans, polychaetes                          | 2.5-3                      | 3-18        |   |       |                       |  |
|                                  |  | Cr  | Crustaceans                                       | 2.5                        | 2-47        |   |       |                       |  |
|                                  |  | Zn  | Crustaceans                                       | 2.5                        | 12-54       |   |       |                       |  |
| <i>Dicentrarchus labrax</i>      | Food chain                                   | Am  | Fish  | 24                         | 4-8         | Mathews & Fisher (2008)                       |       |                       |  |
|                                  |  | Cd  | Fish  | 24                         | 14-31       |   |       |                       |  |
|                                  |  | Co  | Fish  | 24                         | 13-28       |   |       |                       |  |
|                                  |  | Cs  | Fish  | 24                         | 76-82       |   |       |                       |  |
|                                  |  | Mn  | Fish  | 24                         | 24-42       |   |       |                       |  |
|                                  |  | Se  | Fish  | 24                         | 52-76       |   |       |                       |  |
|                                  |  | Zn  | Fish  | 24                         | 28-48       |   |       |                       |  |
|                                  |  | <i>Fundulus heteroclitus heteroclitus</i> | Food chain, Food composition, Subcellular control | As                         | Crustaceans |   | 9     | 9-10                  | Dutton & Fisher (2011), Seebaugh et al. (2005), Goto & Wallace (2009), Mathews & Fisher (2008) |
| Cd                               | Crustaceans, fish, insects, polychaetes      |   |   | 1-13                       | 3-70        |   |       |                       |  |
| Cr                               | Crustaceans, polychaetes                     |   |   | 9                          | 0-4         |   |       |                       |  |
| Hg(II)                           | Crustaceans, polychaetes                     |   |   | 9                          | 10-26       |   |       |                       |  |
| MeHg                             | Crustaceans, fish, insects, polychaetes      |   |   | 1-13                       | 47-96       |   |       |                       |  |
| Po                               | Crustaceans                                  |   |   | 13                         | 25-37       |   |       |                       |  |
| Hg(II)                           | Crustaceans                                  |   |   | 6                          | 25-78       |   |       |                       |  |
| MeHg                             | Crustaceans                                  |   |   | 6                          | 81-98       |   |       |                       |  |
| <i>Gambusia affinis</i>          | Food chain, Trace element pre-exposure       |   |   | MeHg                       | Polychaetes | 1.5   | 52-65 | Leaner & Mason (2002) |  |
|                                  |  |   |   | Hg(II)                     | Crustaceans | 6   | 0-18  |                       |  |
| <i>Ictalurus punctatus</i>       | Food chain, Trace element pre-exposure       | MeHg                                      | Polychaetes                                       | 6                          | 84-94       | Pickhardt et al. (2006)                       |       |                       |  |
|                                  |  | Hg(II)                                    | Crustaceans                                       | 6                          | 84-94       |   |       |                       |  |
| <i>Lutjanus argentimaculatus</i> | Food composition, Ingestion rate             | Cd  | Crustaceans, molluscs                             | 3                          | 4-33        | Xu & Wang (2002), Zhao et al. (2001)          |       |                       |  |
|                                  |  | Cs  | Crustaceans, fish, molluscs                       | 3                          | 82-99       |   |       |                       |  |
|                                  |  | Se  | Crustaceans, molluscs                             | 3                          | 27-60       |   |       |                       |  |
|                                  |  | Zn  | Crustaceans, molluscs                             | 3                          | 13-53       |   |       |                       |  |
| <i>Menidia</i> sp.               | Food composition                             | Cd  | Crustaceans                                       | 0.8                        | 2-4         | Reinfelder & Fisher (1994)                    |       |                       |  |
|                                  |  | Co  | Crustaceans                                       | 0.8                        | 1-3         |   |       |                       |  |
|                                  |  | Se  | Crustaceans                                       | 0.8                        | 25-33       |   |       |                       |  |
|                                  |  | Zn  | Crustaceans                                       | 0.8                        | 4-8         |   |       |                       |  |
| <i>Monodactylus argenteus</i>    | Interspecific companion                      | Co  | Crustaceans                                       | 45                         | 4-5         | Pouil et al. (2017a)                          |       |                       |  |
|                                  |  | Zn  | Crustaceans                                       | 45                         | 14-16       |   |       |                       |  |
| <i>Morone saxatilis</i>          | Allometry, Food chain                        | Ag  | Crustaceans                                       | 13                         | 17-20       | Baines et al. (2002), Mathews & Fisher (2008) |       |                       |  |
|                                  |  | Am  | Crustaceans                                       | 13                         | 5-7         |   |       |                       |  |
|                                  |  | Cd  | Crustaceans, fish                                 | 2-14                       | 19-51       |   |       |                       |  |
|                                  |  | MeHg                                      | Fish  | 2                          | 82-94       |   |       |                       |  |
|                                  |  | Po  | Fish  | 2                          | 12-18       |   |       |                       |  |
|                                  |  | Se  | Crustaceans                                       | 13-14                      | 31-47       |   |       |                       |  |
| <i>Mugilogobius chulae</i>       | Food composition                             | Zn  | Crustaceans                                       | 13-14                      | 21-45       | Zhang et al. (2017)                           |       |                       |  |
|                                  |  | Cu  | Molluscs, polychaetes                             | 3                          | 8-19        |   |       |                       |  |

(Table continued on next page)

Table 1 (continued)

| Fish species                   | Study objectives  | Metal                         | Food   | Duration of depuration (d) | AE (%)               | Reference  |   |       |  |
|--------------------------------|---|-------------------------------|--|----------------------------|----------------------|--|---|-------|--|
| <i>Oryzias melastigma</i>      | Size<br>Sex   | Cu                            | Rotifers   | 28                         | 9–18                 | Guo et al. (2016)  |   |       |  |
| <i>Periophthalmus modestus</i> | Food composition, Interspecific comparison, Salinity  | Cd                            | Crustaceans, polychaetes   | 1–2                        | 2–31                 | Ni et al. (2000), Ni et al. (2005)   |   |       |  |
|                                |   | Cr                            | Crustaceans  | 1–2                        | 1–26                 |  |   |       |  |
|                                |   | Se                            | Crustaceans, polychaetes   | 1–2                        | 32–40                |  |   |       |  |
|                                |   | Zn                            | Crustaceans, polychaetes   | 1–2                        | 1–36                 |  |   |       |  |
| <i>Plectorhinchus gibbosus</i> | Food composition  | Hg(II)                        | Crustaceans, fish  | 1                          | 6–32                 | Wang & Wong (2003)   |   |       |  |
|                                |   | MeHg                          | Crustaceans, fish  | 1                          | 45–98                |  |   |       |  |
| <i>Scatophagus argus</i>       | Interspecific comparison  | Co                            | Crustaceans  | 45                         | 5–6                  | Pouil et al. (2017a)   |   |       |  |
|                                |   | Zn                            | Crustaceans  | 45                         | 23–25                |  |   |       |  |
| <i>Scophthalmus maximus</i>    | Allometry, Food chain, Food composition, Interspecific comparison, Subcellular control, Water pH, Water temperature | Ag                            | Pellet, polychaetes  | 21                         | 0–4                  | Mathews et al. (2008), Pouil et al. (2015), Pouil et al. (2016), Pouil et al. (2017b), Pouil et al. (in press) |   |       |  |
|                                |   | Am                            | Fish   | 21                         | 6–10                 |  |   |       |  |
|                                |   | Cd                            | Fish, pellets  | 21                         | 6–42                 |  |   |       |  |
|                                |   | Co                            | Crustaceans, fish, pellets, polychaetes                            | 21                         | 1–45                 |  |   |       |  |
|                                |   | Cs                            | Fish   | 21                         | 61–65                |  |   |       |  |
|                                |   | Mn                            | Crustaceans, fish, pellets, polychaetes                            | 21                         | 22–46                |  |   |       |  |
|                                |   | Zn                            | Crustaceans, fish, pellets, polychaetes                            | 21                         | 13–33                |  |   |       |  |
|                                |   | Am                            | Fish   | 21                         | 5–7                  |  | Mathews et al. (2008)   |       |  |
|                                |   | Cd                            | Fish   | 21                         | 25–33                |  |   |       |  |
|                                |   | Co                            | Fish   | 21                         | 8–14                 |  |   |       |  |
| <i>Scyliorhinus canicula</i>   | Interspecific comparison  | Cs                            | Fish   | 21                         | 69–77                | Pan & Wang (2016)  |   |       |  |
|                                |   | Mn                            | Fish   | 21                         | 24–30                |  |   |       |  |
|                                |   | Zn                            | Fish   | 21                         | 16–18                |  |   |       |  |
|                                |   | Cs                            | Molluscs   | 2.75                       | 62–83                |  |   |       |  |
|                                |   | Cd                            | Macroalgae   | 2                          | 2–47                 |  | Chan et al. (2003), Zhou et al. (2017)  |       |  |
|                                |   | Cr                            | Macroalgae   | 2                          | 3–24                 |  |   |       |  |
|                                |   | Cu                            | Pellets  | 2                          | 7–20                 |  |   |       |  |
|                                |   | <i>Sebastiscus marmoratus</i> | Food composition   | Zn                         | Macroalgae           |  | 2   | 4–42  | Pan & Wang (2016)                              |
|                                |   |                               |  | Cs                         | Macroalgae, molluscs |  | 2.75  | 40–67 |  |
|                                |   | <i>Siganus canaliculatus</i>  | Food composition, Salinity, Starvation, Trace element pre-exposure | Am                         | Crustaceans, fish    |  | 15–21   | 1–9   | Mathews & Fisher (2008), Mathews et al. (2008) |
| Cd                             | Crustaceans, fish   |                               |  | 15–21                      | 6–50                 |  |   |       |  |
| Co                             | Crustaceans, fish   |                               |  | 15–21                      | 7–23                 |  |   |       |  |
| Cs                             | Crustaceans, fish   |                               |  | 15–21                      | 71–89                |  |   |       |  |
| Mn                             | Crustaceans, fish   |                               |  | 15–21                      | 11–28                |  |   |       |  |
| Se                             | Crustaceans   |                               |  | 15                         | 61–92                |  |   |       |  |
| Zn                             | Crustaceans, fish   |                               |  | 15–21                      | 4–25                 |  |   |       |  |
| Ag                             | Crustaceans   |                               |  | 2                          | 12–41                | Dang & Wang (2010), Long & Wang (2005a), Pan & Wang (2016), Zhang & Wang (2006)                                |   |       |  |
| Cd                             | Crustaceans, fish, molluscs   |                               |  | 1.5–2                      | 2–41                 |  |   |       |  |
| Cs                             | Molluscs  |                               |  | 2–2.75                     | 65–83                |  |   |       |  |
| <i>Siganus fuscescens</i>      | Food composition  | Hg(II)                        | Fish, molluscs   | 2                          | 12–100               | Zhang & Wang (2006)  |   |       |  |
|                                |   | MeHg                          | Fish, molluscs   | 2                          | 52–97                |  |   |       |  |
|                                |   | Se                            | Crustaceans, molluscs  | 1.5                        | 10–63                |  |   |       |  |
|                                |   | Zn                            | Crustaceans, fish, molluscs  | 1.5                        | 1–67                 |  |   |       |  |
|                                |   | Cs                            | Macroalgae, molluscs   | 2.75                       | 40–67                |  |   |       |  |
| <i>Sparus aurata</i>           | Food chain, Interspecific comparison  | Am                            | Crustaceans, fish  | 15–21                      | 1–9                  | Mathews & Fisher (2008), Mathews et al. (2008)   |   |       |  |
|                                |   | Cd                            | Crustaceans, fish  | 15–21                      | 6–50                 |  |   |       |  |
|                                |   | Co                            | Crustaceans, fish  | 15–21                      | 7–23                 |  |   |       |  |
|                                |   | Cs                            | Crustaceans, fish  | 15–21                      | 71–89                |  |   |       |  |
|                                |   | Mn                            | Crustaceans, fish  | 15–21                      | 11–28                |  |   |       |  |
|                                |   | Se                            | Crustaceans  | 15                         | 61–92                |  |   |       |  |
|                                |   | Zn                            | Crustaceans, fish  | 15–21                      | 4–25                 |  |   |       |  |
|                                |   | Ag                            | Crustaceans  | 2                          | 12–41                |  | Dang & Wang (2010), Long & Wang (2005a), Pan & Wang (2016), Zhang & Wang (2006) |       |  |
|                                |   | Cd                            | Crustaceans, fish, molluscs  | 1.5–2                      | 2–41                 |  |   |       |  |
|                                |   | Cs                            | Molluscs   | 2–2.75                     | 65–83                |  |   |       |  |
| <i>Terapon jarbua</i>          | Food composition, Trace element pre-exposure, Subcellular control   | Hg(II)                        | Fish, molluscs   | 2                          | 12–100               | Zhang & Wang (2006)  |   |       |  |
|                                |   | MeHg                          | Fish, molluscs   | 2                          | 52–97                |  |   |       |  |
|                                |   | Se                            | Crustaceans, molluscs  | 1.5                        | 10–63                |  |   |       |  |
|                                |   | Zn                            | Crustaceans, fish, molluscs  | 1.5                        | 1–67                 |  |   |       |  |
|                                |   | Cs                            | Macroalgae, molluscs   | 2.75                       | 40–67                |  |   |       |  |

Warnau et al. 1996). The long-lived component allows the estimating of AE by calculating the y-axis intercept of the 'l' component of the radiotracer ingested with food ( $AE = A_{0l}$ ; Reichle 1967, Fowler & Guary 1977, Miramand et al. 1982, Warnau et al. 1996). In some studies, the depuration of the assimilated fraction of trace elements was shown to be very slow (e.g. Pouil et al. 2015, 2016). When the long-term depuration rate constant ( $k_{el}$ ) is not significantly different from 0, the 'l' component of the exponential model can be simplified and replaced by a constant (e.g. Pouil et al. 2015, 2016) and the equation becomes:

$$A_t = A_{0s} \times e^{-k_{es}t} + A_{0l} \quad (4)$$

with  $A_{0l} = AE$ .

This method requires that the fish be depurated for a sufficiently long period of time to get an accurate determination of the slope of the slowest depurating compartment. Usually, the depuration of the fish is followed for several weeks (Table 1). Because all the excretion processes (urinary, branchial and biliary) are taken into account, it is the most robust method to accurately determine AE (see next subsection, 'How the duration of depuration influences AE determination').

From a mechanistic point of view, 3 different phases occur during the depuration of an element. The first phase, usually a few hours after the feeding, is very rapid and corresponds to the passage of the ingested food from the stomach to the intestine where the absorption process occurs (Baines et al. 2002, Dutton & Fisher 2011), i.e. the 's' component described under Eq. (3). The second phase, usually in the first week of depuration, is dominated by the occurrence of the absorption and excretion processes (Baines et al. 2002, Dutton & Fisher 2011, Pouil et al. 2016). During phases 1 and 2, as shown by Pouil et al. (2017a), almost all the trace elements ingested are distributed in the stomach and the intestine. Then, the third phase reflects the physiological turnover from the slowest depurating compartment after absorption and excretion (Wang & Fisher 1999). The loss of trace elements during this phase is reduced and the body burden of trace elements is stabilizing. Phases 2 and 3 are usually difficult to resolve using depuration biokinetic models; in a 2-component exponential model, these phases are included in the 'l' component.

#### How the duration of depuration influences AE determination

From a practical point of view, the duration of the follow-up period of the depuration, as defined by

the experimenters, is decisive to 'catch' these biological processes. As explained above (see 'The need to clearly define the concept of AE'), GTT can be used to estimate the duration needed for the experiments in order to determine AE accurately. Some authors estimate the GTT by the frequent collection and the radio-counting of faeces subsequently to single-feeding, and thus GTT ends when the last radioactive faeces have been collected (e.g. Ni et al. 2000). Thus, the duration of depuration is chosen to cover the GTT (in general, from 24 to 72 h in fish; e.g. Xu & Wang 2002, Van Campenhout et al. 2007, Goto & Wallace 2009). When the depuration follow-up is made over a period close to GTT (the 'short-term' approach), AE is determined at a given time and expressed as a percentage of the trace element retained. This approach does not allow taking into consideration the third phase of the depuration kinetics (i.e. when physiological turnover occurs after absorption and excretion), as is usually achievable when the duration of depuration extends over several weeks (i.e. the 'long-term' approach).

In order to compare AEs obtained using both the 'short-term' and 'long-term' approaches, statistical comparison (Wilcoxon-Mann-Whitney non-parametric test) was done on data provided by Pouil et al. (2017b, their supplementary material) for  $^{54}\text{Mn}$  remaining activities at different times throughout a 21 d depuration in turbot *S. maximus* fed with compounded pellets (Fig. 1). Remaining activities were stable from Day 2 (i.e. <24 h after GTT) up to Day 21 after the beginning of the depuration ( $p > 0.05$ ). However, statistical comparison between the individual AE estimated as the percentage of remaining activity after 2 d ('short-term' approach) and the individual AE obtained by fitting a model using data collected over 21 d ('long-term' approach) indicated a significant overestimation of AE by the 'short-term' approach ( $p = 0.04$ ). This example shows that, in a given dataset, the 'short-term' and 'long-term' approaches may lead to different AE estimations. Such bias can be avoided using a sufficiently long period of depuration that encompasses both the absorption and the excretion processes and allows an accurate delineation of the AE. In the 'short-term' depuration approach, part of the excretion processes occurring during the last phase of the depuration are assumed to be negligible, which is obviously not correct. Therefore, this approach should be only considered after a careful investigation of the depuration processes in the given experimental conditions.

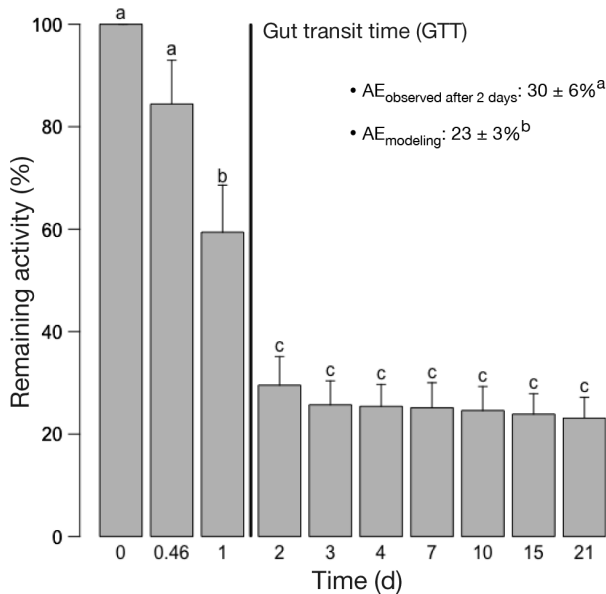


Fig. 1. Remaining activities of  $^{54}\text{Mn}$  during 21 d depuration in turbot *Scophthalmus maximus* ( $n = 12$ ) fed radiolabelled pellets. Data from Pouil et al. (2017b, their supplementary material). For comparison, assimilation efficiency (AE) observed after 2 d of depuration ('short-term' approach) and AEs estimated using kinetic modelling ('long-term' approach) are indicated. Error bars are  $\pm$ SD. Different lower-case letters indicate significant differences ( $p < 0.05$ ). Note: x-axis scale is not linear

## REVIEW OF TRACE ELEMENT AE STUDIES IN FISH

### AE related to trace elements and depuration duration

Fig. 2 shows reported ranges of AEs for different essential (i.e. metabolically required) and non-essential (no known biological role) elements in fish. This overview of results from 35 experimental studies reveals that the findings regarding trace element AE are overall similar regardless of the method of determination (i.e. 'short-term' and 'long-term' approaches; Fig. 2A,B). However, using Zn, one of the most studied elements, analysis of the coefficients of variation (estimating dispersion of values from the average) for the AE values reveals that the 'short-term' approach leads to higher AE variability than the 'long-term' approach. This analysis provides an overall picture of AE variability according to the approach adopted for its determination. These findings, however, must be nuanced by the fact that other experimental factors that can also affect AE variability (e.g. objectives of the study, number of organisms, etc.) are not taken into account.

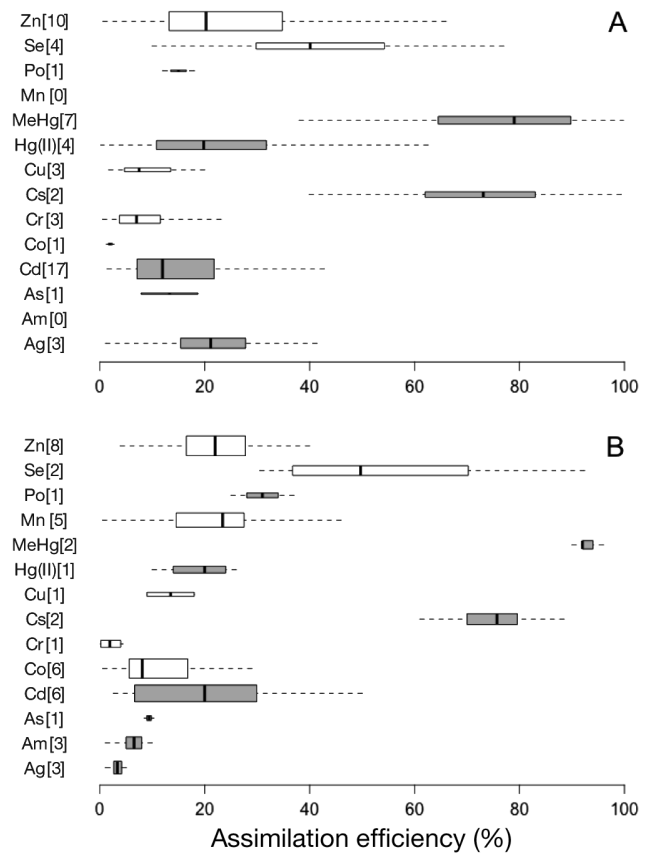


Fig. 2. Comparison of assimilation efficiency (AE) values of essential (white bars) and non-essential (grey bars) elements assessed in fish, in (A) 'short-term depuration' and (B) 'long-term depuration' experiments. Height of the boxes is proportional to the number of observations; thick black line represents the median; dashed line represents the range. Extreme values are not represented. Number of studies are enclosed in square brackets. Data extracted from the literature are detailed in Table 1. MeHg: methylmercury

Non-essential elements (Ag, Am, As, Cd, Cs, Hg(II), Po) and a compound (methylmercury, MeHg) are the most studied with, in particular, Cd AE values available for 15 species of fish (Fig. 2, Table 1). Among the 6 reported essential elements, Co, Cu, Cr, Mn, Se and Zn, the latter element is the one with the most AE values available (>180 values, expressed as means  $\pm$  SD). Analysis of the AEs for the different trace elements shows that there is no obvious relationship between the essential character of a trace element and its assimilation by the fish, in contrast to what has been observed in invertebrates (Wang & Fisher 1999). Interestingly, MeHg and Cs, which are non-essential, are very efficiently assimilated by fish. The high AE values explain for a large part why MeHg and Cs biomagnify in aquatic food webs in both freshwater and marine ecosystems (e.g. Garnier-Laplace et al. 2000,



Zhao et al. 2001, Harmelin-Vivien et al. 2012, Lavoie et al. 2013, Pan & Wang 2016). Among the most efficiently assimilated elements, Se is an essential trace element known to have an antagonistic action with Hg in aquatic organisms (Belzile et al. 2006). Field investigations have shown that high Se concentrations may force a preferential assimilation of this element over Hg through a competitive adsorption on binding sites. The occurrence of Se at high concentrations may also restrict the solubility and bioavailability of Hg to aquatic organisms or reduce its methylation in freshwater ecosystems (Cuvin-Aralar & Furness 1991, Belzile et al. 2006, Yang et al. 2008). To the best of our knowledge, no experimental study has investigated such an effect in fish.

### Factors influencing trace element AEs in fish

In theory, AE can be influenced by both abiotic and biotic factors, because both potentially affect fish physiology and bioavailability of, or bioaccessibility to, trace elements. Biotic factors have been the most studied in the literature (Fig. 3). The AE of trace elements in fish depends on the relationship between prey and their predators (Fig. 3). Thus, it is possible to distinguish 2 types of biotic factors: those related to prey and those related to predators.

Numerous studies have investigated the influence of food quality (type of natural prey and compounded food) on AE in fish. It has been shown for example that, in the same predator species, AEs can be very

different depending on the type of food ingested (e.g. Dutton & Fisher 2011, Wang et al. 2012, Pouil et al. 2016). Using a mechanistic approach, some authors have studied the factors related to the prey (bivalves and oligochaetes) that could explain these differences. In particular, based on studies initiated with invertebrates (crustaceans; Wallace & Lopez 1996, Wallace & Luoma 2003), the relationship between the subcellular fraction of trace elements in food and the AE observed in predators has been investigated in several species (Zhang & Wang 2006, Dang & Wang 2010). However, the results showed contrasts. Some studies highlighted a positive relationship between the cytosolic fractionation of Cd, MeHg, Se and Zn in the prey and the AEs of these elements in different species of fish fed zooplankton, molluscs or selected fish tissues (Zhang & Wang 2006, Dang & Wang 2010). However, more recently, Pouil et al. (2016) found no obvious relationship for essential elements (Co, Mn and Zn) in juvenile turbot *Scophthalmus maximus* fed more complex food matrices (complex pluricellular natural whole prey).

Interspecific comparisons of trace element AEs have also been made (e.g. Ni et al. 2000, Pouil et al. 2017a). The differences observed were often related to the trophic ecology of the organisms or their phylogeny. The influence of predator size (i.e. allometry) on AE was also investigated in black seabream *Acanthopagrus schlegeli* (Zhang & Wang 2007). In that study, Cd AE was independent of body

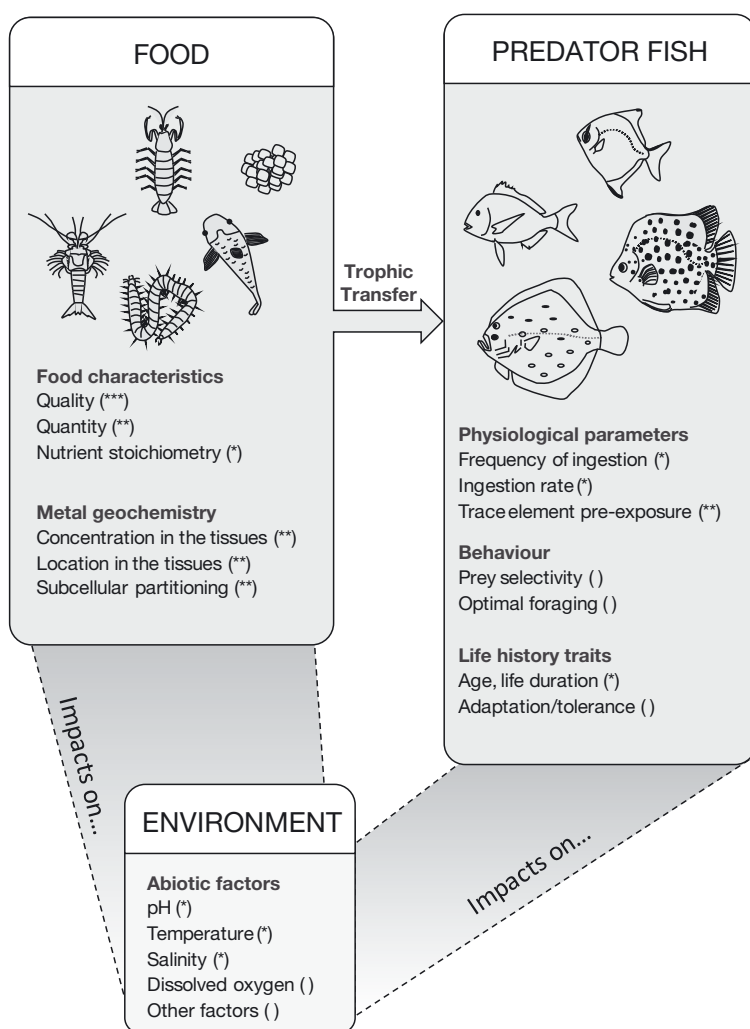


Fig. 3. Processes controlling the assimilation efficiency of metals in predator fish. Stars in brackets indicate that the process has been studied in the literature, with the number of stars proportional to the quantity of information available in the literature. Absence of stars: the process has not yet been investigated

size, whereas Se and Zn AE increased with predator size. Guo et al. (2016) showed that Cu AE increased during development from larvae to adults in the fish model *Oryzias melastigma*.

Regarding the feeding behaviour of predators, although this parameter appears to be important in the understanding of trace element assimilation, there are still only a few studies that have tackled this aspect. Among these, Van Campenhout et al. (2007) demonstrated in common carp *Cyprinus carpio* that the frequency and rate of ingestion have a significant impact on the AE of Cd and Zn. Similar findings were reported for the AE of Cu in black seabream juveniles (Guo et al. 2015).

The influence of water temperature on AE has also been investigated (Van Campenhout et al. 2007). Those authors observed that decreasing the temperature from 25 to 15°C did not influence Cd AE, whereas a significant decrease in Zn AE was found. The influence of trace element pre-exposure in the environment has also been considered in a few studies. It was for instance shown that Ag AEs were higher in waters highly contaminated by this element (Long & Wang 2005b, Boyle et al. 2011). However, no effect was observed for Cd or Zn (Zhang & Wang 2005, Boyle et al. 2011).

Besides temperature or element concentrations, there is still a lack of knowledge regarding the possible effects of other abiotic factors on AE in fish. The limited amount of information available might result from the non-obvious connection between these factors and the trophic transfer of metals. Abiotic factors such as salinity or pH are generally recognized for their ability to influence trace element speciation in the water and thus their impact on uptake from the gills rather than AEs from the diet. These factors can however also influence fish physiology, with possible indirect effects on metal uptake through the food.

Salinity, which is a key parameter in brackish and marine environments that influences both bioavailability of trace elements and fish physiology, has been investigated in fish (Ni et al. 2005, Zhou et al. 2017). Ni et al. (2005) found no significant differences in Cd, Se and Zn AEs in the mudskipper *Periophthalmus modestus* acclimated from 10 to 30 psu, whereas Zhou et al. (2017) found that Cu AEs measured in the white-spotted spinefoot *Siganus canaliculatus* decreased from 33 to 10 psu and increased at 5 psu. Furthermore, those authors found that high dietary Cu pre-exposure reduced its AE regardless of the salinity (Zhou et al. 2017).

Recently, environmental pH, known to influence the digestive physiology of fish (Zhang & Wang 2006, Dang & Wang 2010), was considered to explore the

possible effects of ocean acidification on stomach pH and the assimilation of essential elements in clownfish *Amphiprion ocellaris* (Jacob et al. 2017) and turbot *S. maximus* (Pouil et al. 2017b). Both studies showed no significant effect of environmental pH on the AE of Ag, Co and Zn.

## CONCLUSION

AE is a key parameter in the trophic transfer of trace elements in fish and is therefore widely investigated in ecotoxicology and aquaculture research. Despite its extensive use, there are still divergences in the definition of the AE concept, which may affect its experimental determination. We have provided here a critical analysis of the methods used to determine AE in fish in order to provide guidance for future studies. Among the 35 experimental studies of trace element AE in fish we found in the literature, the influence of environmental variables in the trophic transfer of these elements has received little attention. This research topic continues to offer exciting and challenging scientific questions for ecotoxicology and fish nutrition research.

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