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基于耳石核心氧同位素 SHRIMP 分析研究青海湖裸鲤繁殖特征

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摘要: 青海湖裸鲤是中国重要的内陆珍稀鱼种，在青海湖湖泊生态系统中起着核心作用。繁殖环境是鱼类种群延续的关键因素，能获取青海湖裸鲤的繁殖环境参数和明确最佳产卵场，对于保护和扩大其渔业资源量也非常重要。本文尝试利用耳石的微区原位氧同位素组成分析青海湖裸鲤的繁殖特征，利用 SHRIMP Ⅱ离子探针测定 5 尾青海湖裸鲤耳石微区原位 δ^{18}O 组成，沿着最长生长轴到边缘打点，束斑直径大约 25μm，束斑深度约 2~3μm。分析结果表明，裸鲤耳石的 δ^{18}O 值变动范围分别是−4.88‰~3.46‰、−0.28‰~3.91‰、−1.43‰~2.94‰、−1.81‰~3.35‰，并且耳石间歇带的 δ^{18}O 值高于成长带。耳石间歇带是裸鲤在湖水中形成，而成长带是在河水中形成，上述结果与青海湖湖水的 δ^{18}O 值显著高于河水的 δ^{18}O 值一致，因此记录了裸鲤的洄游行为。核心区域差异性的 δ^{18}O 值则反映了裸鲤的产卵地和水温状况，表明有的裸鲤在水温较低的河口产卵孵化，有的在水温较高的河流上游产卵孵化。与其他样品不同的是，其中 1 尾裸鲤耳石的 δ^{18}O 变动范围是−9.36‰~−5.21‰，表明该裸鲤固定在河流里生长繁殖，不发生洄游行为。这一发现为进一步优化青海湖裸鲤的人工繁殖和利用耳石化石探究青海湖水环境演变提供了研究基础。

关键词: 青海湖裸鲤；产卵地；耳石；氧同位素；SHRIMP；水温

要点:

(1)利用 SHRIMP Ⅱ离子探针测定耳石微区原位 δ^{18}O 组成，实现了耳石高分辨率微量样品采集和分析。

(2)利用耳石核心的 δ^{18}O 组成揭示了青海湖裸鲤有 3 种繁殖群体类型。

(3)基于耳石 δ^{18}O 值与水温显著相关，示踪青海湖裸鲤孵化的温度史。

中图分类号: TQ123.1；S124+2 **文献标识码:** A

青海湖裸鲤,俗称“湟鱼”,是青海湖中唯一的经济鱼类,处于湖区鱼鸟共生生态系统的核区地位,每年 5~8 月份洄游到布哈河、沙柳河、黑马河、泉吉河、哈尔盖河产卵繁殖[1]。由于捕捞强度大、气候干旱、产卵河道断流等原因导致其资源量急剧下降,产量曾由 20 世纪 60 年代的 2.8 万吨降低至 90 年代的 2263 吨,鉴于其重要的生态地位,恢复和保护青海湖裸鲤资源十分迫切[2]。青海省自 1982 年开始实施封湖育鱼,同时在青海湖周边先后建立了原种保种基地和人工增殖放流站,截至 2022 年青海湖裸鲤资源

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量提高到 10.85 万吨, 是保护初期的 42 倍。而繁殖环境是鱼类种群延续的关键因素, 不同鱼类对产卵场的环境参数需求也不同, 如河流、水温、光照、底质和水温等。能获取青海湖裸鲤的繁殖环境参数和明确最佳产卵场, 对于保护和扩大其渔业资源量也非常重要。目前仅见周杨浩等[3] 通过室内人工模拟的方法探讨青海湖裸鲤自然繁殖的环境需求。因此, 研究裸鲤的繁殖环境对自然栖息地保护与修复工作具有重要意义。

耳石是硬骨鱼体内的碳酸钙矿物, 兼具听觉和平衡作用, 被称为时间和水环境的“记录器”[4]。耳石的微化学分析能够揭示鱼类的生活史和水环境变化, 尤其耳石核心区形成于孵化初期, 其微化学组成可以反映鱼类出生地的水化学特征, 能够解决鱼类群体识别问题[5-7]。其中耳石中的氧同位素组成与温度密切相关[8], 在正常的盐度条件下, 1‰的 δ^{18}O 变化对应于大约 5℃ 海水温度的变化[9]。例如, 太平洋蓝鳍金枪鱼(*Thunnus orientalis*)从西太平洋(WPO)到东太平洋(EPO)之间跨太平洋迁移, 其耳石中较高的 δ^{18}O 值反映了迁徙过程中遇到的低水温[10]。类似的, 在淡水环境和海洋环境之间洄游的大麻哈鱼(*Oncorhynchus keta*)耳石核心中较低的 δ^{18}O 值指示了中国的大麻哈鱼也是溯河洄游产卵, 孵化后返回海洋育肥[11]。袁威等[12] 采集并测试了黄海、渤海小黄鱼耳石中的 δ^{13}C 和 δ^{18}O 值, 首次将黄海南部种群细分为黄海南部离岸种群和黄海南部沿岸种群, 且 2 种群之间很少有站位的交叉现象, 经过聚类分析得到黄海、渤海秋季小黄鱼群体总体判别成功率为 82.6%。Tatsuya 等[13] 对沙丁鱼(*Sardinops sagax*)耳石 δ^{18}O 和微观结构研究, 探讨其保育栖息地温度和早期生长速度的关系。可见, 耳石中的 δ^{18}O 组成为鱼类种群辨识、洄游迁移、产卵地环境探究提供了方法手段。

青海湖作为气候环境变化敏感区, 利用介形虫、沉积物氧同位素组成研究其历史时期的气候变化和湖泊演变的成果较多[14-15]。而关于青海湖裸鲤的研究主要集中在形态特征、生长特征、遗传性状等方面[16-18]。本文课题组前期测定了青海湖裸鲤耳石中的微量元素如 Sr/Ca 比值, 发现耳石轮纹中 Sr/Ca 比值的高低变化指示了裸鲤的洄游行为[19], 由于耳石成长带中的 Sr/Ca 比值呈现出频繁的波动, 可能与河水与微耳石之间 Sr 的分配系数不同于湖水以及鱼体的生理代谢相关。耳石中的 δ^{18}O 组成主要受水温影响, 尤其耳石核心的微化学组成能够指示其孵化产卵场的环境。基于前期的研究成果, 本文利用 SHRIMP Ⅱ 离子探针测定青海湖裸鲤耳石中 δ^{18}O 的组成, 结合青海湖的湖水和河水的水化学组成, 探讨其最佳洄游产卵地和环境条件, 为进一步揭示青海湖裸鲤的生活史提供有效信息, 为青海湖的渔业资源管理和渔业科学研究奠定基础。

1　研究区地理位置和自然条件

青海湖地处青藏高原东北部, 长 105km, 宽 63km, 湖面海拔 3196m, 西边是柴达木盆地, 东边是日月山, 北边是大通山, 南边是青海南山, 它不仅连接青海省的东部、西部和南部, 也是通往甘肃河西走廊、西藏自治区和新疆维吾尔自治区的重要通道。青海湖状似梨形, 截至 2021 年 9 月底, 青海湖水体面积为 4625.6 平方公里。如图 1 所示。

青海湖流域气候类型属于高原大陆性气候, 地处西风带、高原季风、东亚季风的交错带, 光照充足、阳光强烈, 干湿季节分明。青海湖的日照时数大多数都超过 3000h, 全年辐射总量在 171.461 ~ 106.693 千卡/平方厘米·年。湖区东面和南面温度稍高, 西面和北面稍低, 平均最高气温在 6.7 ~ 8.7℃, 平均最低气温在−6.7 ~ 4.9℃, 极端最高气温是 25℃, 极端最低气温是−31 ~ −33.4℃。湖区年降水量少, 蒸发量远远超过降水量。青海湖因含有少量无机盐类, 湖水冻结的温度比 0℃ 稍低[20]。

2　实验部分

2.1　样品采集

采集青海湖生长的现代野生裸鲤 5 尾, 样品编号是 1 号~5 号。采样点位于布哈河口水文站。捕捞当天对裸鲤的体长进行测量、称体重并对其拍照后进行解刨, 使用解刨刀和镊子取出鱼内耳中的三对耳石, 将其放进离心管并加入浓度为 10% 的双氧水溶液, 用超声波振荡器清洗 2min; 抽出双氧水溶液, 加入蒸馏水, 混合后用超声波振荡 2min; 以上动作重复三次, 以彻底清除耳石上的有机组织和双氧水溶液。在烘箱中放入清洗干净的耳石, 对其进行烘干留下备用。青海湖裸鲤的三对耳石照片[19] 如图 2 所示。微耳石(图 2a)形似椭圆, 矿物组成是纯文石; 星耳石(图 2b)形近圆形, 矿物成分主要是球霰石, 还有少量的方解石; 矢耳石(图 2c)形似针状易碎, 不易摘取完整样品, 其主要成分是文石, 含有少量球霰石, 文石组分对环境更为敏感[19], 因此本文选用微耳石进行 δ^{18}O 测试。

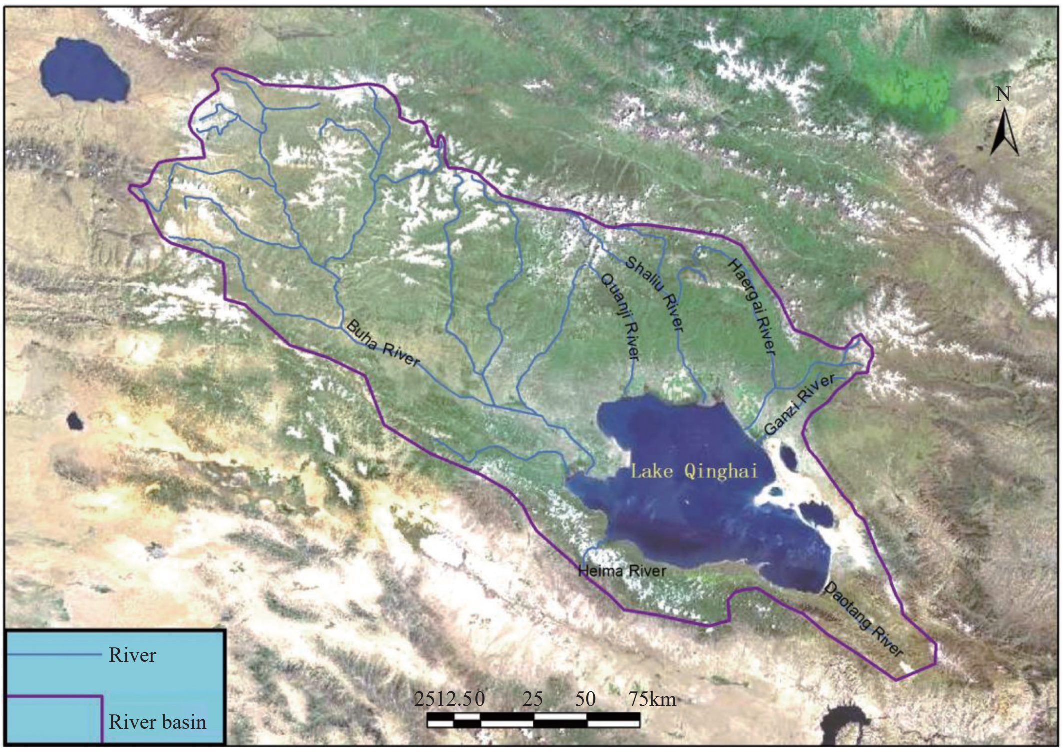
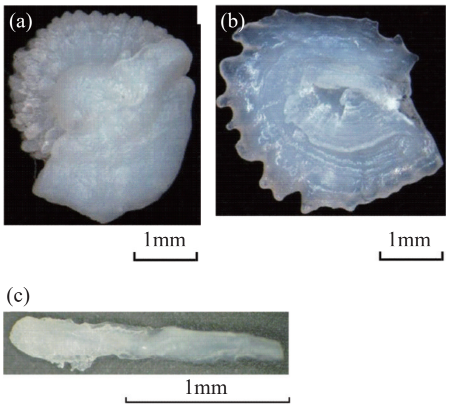


图1　青海湖及其流域图

Fig. 1　Map of Qinghai Lake and its river basin.



a—微耳石； b—星耳石； c—矢耳石。

图2　青海湖裸鲤的三对耳石

Fig. 2　Three pairs of naked carp otoliths (a—lapillus, b—asteriscus, c—saggita).

2.2　氧同位素分析测试方法

微耳石的微区原位氧同位素分析测试在澳大利亚国立大学地球科学学院用 SHRIMP Ⅱ离子探针完成。微耳石经过树脂包埋、打磨、抛光、清洗、烘干等操作后，表面喷涂高纯度铝，转移到 SHRIMP Ⅱ离子探针，参照该实验室 Long 等[21-22] 描述的详细步骤测试。该仪器配备 15kV、~3nA Cs^+离子源，在电压作用下离子从离子枪内射出，被聚焦后照射在样品表面激发 200~250pA 的二次离子。法拉利杯同时接收$^{16}O^-$和$^{18}O^-$，并用 Keithley 642 静电计测量出离子数。每次测试包含 3min 的预烧以保证二次离子的同位素组成更稳定，以及 10 次或 14 次(10s/次)的$^{18}O/^{16}O$ 比值的计算时间。使用的标准品是 NBS18 和 NBS19[23]，保证仪器测试的准确性和精确性，精确度为 0.1‰~0.2‰，标准偏差约为 0.3‰。测试中每完成 5 个样品，都会测一次标准物质。离子探针的打点起始位置是耳石的核心处，沿着最长生长轴一直到边缘打点，束斑直径大约 25μm，束斑深度约 2~3μm，打点测试如图 3 所示。

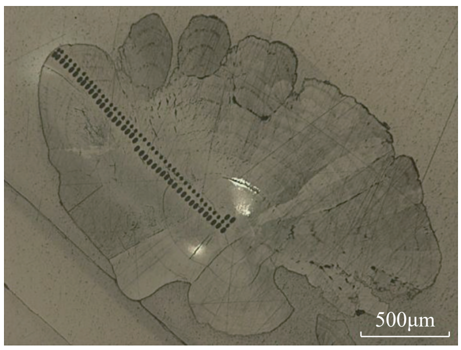


图3　青海湖裸鲤微耳石离子探针测试样点

Fig. 3　Sample points of otolith in naked carp by ion probe.

3　结果

3.1　青海湖裸鲤的年龄确定

将 5 尾裸鲤的微耳石在研磨机上反复研磨, 直至获得最佳轮纹照片, 如图 4 所示。微耳石核心区域致密, 其外是年轮排列区, 窄的间歇带与宽的成长带相间排列, 由于湖水相较于河水温度低且食物缺乏, 因此间歇带是裸鲤在湖水中形成, 而成长带是裸鲤在河水中形成[19]。熊飞等[24]通过各种年龄鉴定材料比较研究了青海湖裸鲤的年轮特征, 结果得到微耳石磨片是高龄裸鲤较为可靠的年龄鉴定材料。因此, 由两位计数人员将各自读取的微耳石的轮纹数进行对比, 确定每尾裸鲤的最终年龄, 统计信息如表 1 所示。本次研究获取的裸鲤体长范围是 30~42cm, 平均体长为 34.4cm, 体重范围是 302~590g, 平均体重为 426.4g, 年龄范围是 7~12 龄。

3.2　青海湖裸鲤的微耳石生长轴的 δ^{18}O 组成

选取的 5 尾裸鲤微耳石生长轴的 δ^{18}O 值如图 5 所示。1 号~5 号样品耳石的 δ^{18}O 测定平均值分

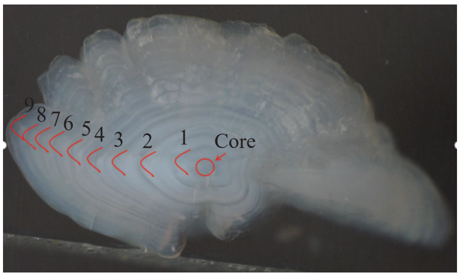


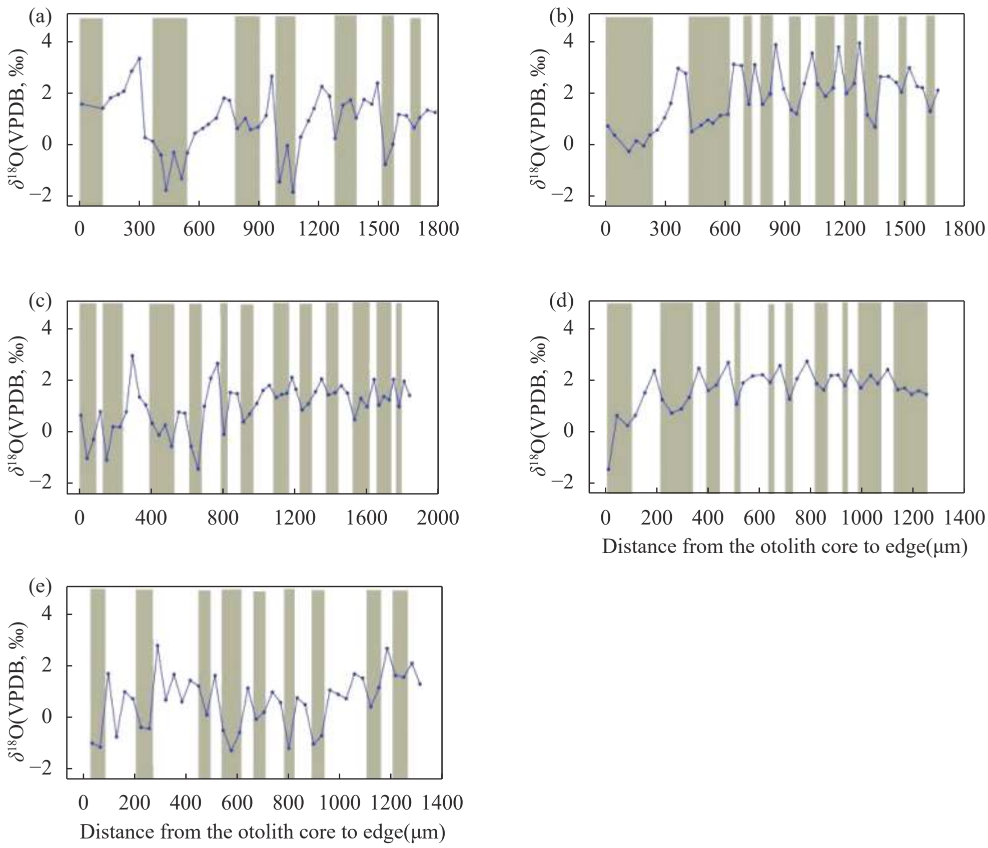
图4　样品 5 号微耳石的年轮照片

Fig. 4　Photo of the annual rings of No.5 otolith.

表 1　5 尾裸鲤的体长、体重和耳石年轮统计结果

Table 1　Statistical results of body length, weight and otolith rings of 5 naked carp.

样品编号 Sample No.	裸鲤体长 Length (cm)	裸鲤体重 Weight (g)	年轮计数 Annual ring count
1	30.0	302	7
2	35.0	450	10
3	42.0	590	12
4	33.0	440	10
5	32.0	350	9



(a)~(e)分别对应 1 号~5 号耳石样品。灰色条带是耳石轮纹中的成长带。

图5　青海湖裸鲤微耳石从核心到边缘 δ^{18}O 的组成

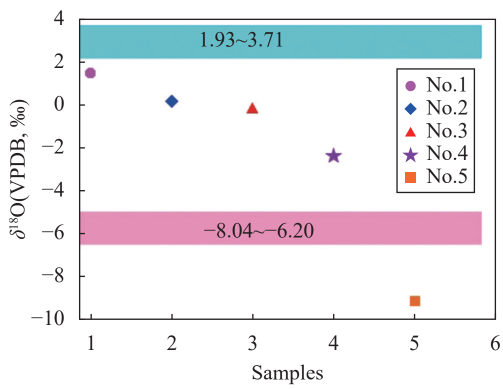
Fig. 5　Composition of δ^{18}O from core to edge of otolith in naked carp. (a)~(e) in the figure correspond to otolith samples 1−5, respectively. The gray band is the incremental band in the otolith.

别是 1.29‰、1.79‰、0.99‰、0.93‰、−7.41‰，变化范围分别是−4.88‰~3.46‰、−0.28‰~3.91‰、−1.43‰~2.94‰、−1.81‰~3.35‰、−9.36‰~−5.21‰。在温度分别为 11℃ 和 17℃ 的条件下饲养欧洲鲽的 $\delta^{18}O$ 值变化范围是 0.2‰~1.9‰，耳石的 $\delta^{18}O$ 受到水温的显著影响，但是不受饲喂水平影响，且饲喂水平与温度之间无显著性的协同效应[8]。欧洲鲽的 $\delta^{18}O$ 值与本文 1 号~样品 4 号中较高的 $\delta^{18}O$ 值比较接近，这是裸鲤有部分时间生活在盐度较高且水温偏低的湖水中的结果。四川裂腹鱼与青海湖裸鲤同属裂腹鱼亚科，用淡水养殖的四川裂腹鱼微耳石 $\delta^{18}O$ 平均值为−9.4‰[25]，这与 5 号裸鲤的 $\delta^{18}O$ 值很接近，说明样品 5 号裸鲤一直生活在淡水中。图 5 中灰色条带是耳石的成长带，成长带之间是间歇带，1 号~5 号样品裸鲤的 $\delta^{18}O$ 值都呈现出成长带较低和间歇带较高的差异，这是因为裸鲤的耳石在春夏季节形成成长带，秋冬季节形成间歇带，春夏季的水温显著高于秋冬季，且耳石的 $\delta^{18}O$ 值与水温成反相关。对 5 尾裸鲤微耳石的 $\delta^{18}O$ 值进行单因素方差分析，1 号、3 号和 4 号裸鲤的 $\delta^{18}O$ 值之间差异性不明显，$P>0.05$，2 号和 5 号裸鲤与其他三尾裸鲤的 $\delta^{18}O$ 值差异明显，$P<0.05$。

通过 Cui 等[26] 和 Liu 等[27] 研究已知青海湖湖水的 $\delta^{18}O$ 值范围是 1.93‰~3.71‰，平均值是 2.43‰，周围河流的 $\delta^{18}O$ 值范围是−8.04‰~−6.20‰，其中，布哈河的 $\delta^{18}O$ 平均值是−6.15‰，沙柳河的 $\delta^{18}O$ 平均值是−7.21‰，泉吉河的 $\delta^{18}O$ 平均值是−5.98‰，黑马河的 $\delta^{18}O$ 平均值是−7.31‰。这是因为不同类型水体 $\delta^{18}O$ 值差别很大，湖水由于强蒸发其 $\delta^{18}O$ 值明显大于地表河水[28]。将湖水和河水的 $\delta^{18}O$ 值与 5 尾裸鲤的 $\delta^{18}O$ 值对比分析，可以看出 1 号~4 号裸鲤的微耳石中记录了湖水和河水的 $\delta^{18}O$ 信号，说明它们都在湖水和河水之间洄游，而 5 号裸鲤的微耳石中仅记录了河水的 $\delta^{18}O$ 信号，说明该裸鲤一直在河水中生长发育，即“坐水鱼”。

3.3 青海湖裸鲤微耳石核心 $\delta^{18}O$ 组成

1 号~5 号耳石核心区的 $\delta^{18}O$ 平均值分别是 1.51‰、0.20‰、−0.22‰、−2.38‰、−9.14‰，变动范围分别是 1.43‰~1.59‰、−0.28‰~0.7‰、−1.02‰~0.64‰、−4.88‰~−0.74‰、−9.36‰~−8.96‰。将耳石核心的 $\delta^{18}O$ 平均值与湖水、河水的 $\delta^{18}O$ 组成进行对比，如图 6 所示。由于耳石核心区域是裸鲤孵化初期形成，因此记录了出生时的水环境状况。从分布来看，1 号~3 号裸鲤核心区域的 $\delta^{18}O$ 值更接近湖水，说明它们的产卵地在河口附近，该水体混合了湖水和河水，水温较低，可能 5 月下旬就开始了洄游产卵，4 号裸鲤核心区域的 $\delta^{18}O$ 值更接近河水，说明其产卵地远离湖水且水温较高，5 号裸鲤则一直生活在河水中，产卵时水温较高。可以看出，青海湖裸鲤的生活习性并不固定，有的在河口产卵孵化，有的在河流上游产卵孵化，有的会在河湖之间洄游产卵，还有一小部分裸鲤固定在河流繁育，这可能与当地河流在秋冬季断流有关系，部分洄游到河流上游产卵的鱼体因河流断流就一直停留在河流中繁育。



图中耳石核心的 $\delta^{18}O$ 值是核心区域的平均值，蓝色条带表示湖水的 $\delta^{18}O$ 范围，粉色条带表示河水的 $\delta^{18}O$ 范围。

图6 青海湖裸鲤微耳石核心、青海湖湖水及主要河流的 $\delta^{18}O$ 组成的分布图

Fig. 6 Distribution graph of $\delta^{18}O$ composition of the naked carp otolith core, lake water and major rivers. The $\delta^{18}O$ values of the otolith core in the figure are the average values of the core area, with blue bands indicating the $\delta^{18}O$ range of the lake and the pink bands indicating the $\delta^{18}O$ range of the river.

4 讨论

4.1 耳石的 $\delta^{18}O$ 值与水温关系解析

青海湖裸鲤耳石不仅能够记录年龄，还能够记录鱼类生活的水环境和鱼类的生活史等。水体 $\delta^{18}O$ 值和耳石形成时鱼类在水中生活的温度决定着鱼类耳石的 $\delta^{18}O$ 值。前者的 $\delta^{18}O$ 值与降水量和蒸发量的比值、径流量和降水的 $\delta^{18}O$ 值相关，后者与耳石的 $\delta^{18}O$ 值呈反相关。鱼类的栖息生活环境可以用耳石的 $\delta^{18}O$ 值进行推断，周围水环境和文石矿物之间达到或者几乎达到同位素之间的平衡，所以 $^{16}O/^{18}O$ 同位素之间的分馏与水体的温度有着紧密的关系，一般呈现负相关[29-30]。对于海水鱼来说，在正常的

盐度条件下, 1‰的 $\delta^{18}O$ 变化对应于大约 5℃ 海水温度的变化[9]。关于淡水鱼耳石 1‰的 $\delta^{18}O$ 变化对应水温的变化关系尚未确立。

在鱼类繁殖过程中, 水温、水流、水深、河床质和光照等环境参数是普遍考虑的要素。一般来说, 鱼类的繁殖活动不会因某一单因素环境因子而被决定, 其繁殖特定需求往往是多种环境因素。当青海湖周围淡水支流水温达到 6~16℃ 这个阈值后, 青海湖裸鲤亲体开始大规模生殖洄游, 本文获得的 5 尾裸鲤耳石核心区 $\delta^{18}O$ 平均值差异显著, 一方面是由于产卵地不同具有差异性的 $\delta^{18}O$ 组成, 另一方面则是产卵时间不同, 导致的水温差异记录在了耳石 $\delta^{18}O$ 中。到底水中的 $\delta^{18}O$ 组成和水温变化对裸鲤耳石中的 $\delta^{18}O$ 值贡献是多少, 我们暂时无法量化, 这需要通过人工控制实验进一步验证。例如, Nakamura 等[31] 采用 6 种不同温度 16.3℃、17.6℃、18.3℃、20.0℃、24.0℃ 和 26.5℃ 培养鲐鱼幼鱼, 并测定 $\delta^{18}O$ 值, 确定了饲养水温与鲐鱼耳石 $\delta^{18}O$ 之间的线性关系。Willmes 等[32] 采用不同盐度(8.75‰、5.28‰和 4.06‰)和不同水温(16.4℃、16.7℃、18.7℃和 20.5℃)条件下饲养越洋公鱼 360 天, 进而重建该鱼的热生活史。Wang 等[33] 评估了烹饪行为是否会导致耳石中碳酸盐的进一步同位素分馏, 结果表明耳石样品的 $\delta^{18}O$ 值在煮熟和未煮熟之间高度一致, 表明烹饪过程对耳石同位素值没有或影响很小。

耳石能够完整地保存在沉积物中, 因此化石耳石常用来开展古气候、古生态和古地理等方面的研究。例如, Wurster 等[34] 分析了采集自伊斯特曼考古地的淡水石首鱼矢耳石高分辨率的 $\delta^{18}O$ 值, 推断出美国东部内陆全新世的气候变化情况; Andrus 等[35] 用保存完整的秘鲁雅首海鲶化石耳石的 $\delta^{18}O$ 值推算出中全新世秘鲁海的海水表面温度; Surge 等[36] 分析了墨西哥拟海鲶的现代和大约公元 2~3 世纪化石耳石的 $\delta^{18}O$ 值, 恢复的冬季温度与现代非常相似。Long 等[21] 分析了澳大利亚蒙哥湖沉积物中耳石的 $\delta^{18}O$ 值, 其 $\delta^{18}O$ 值的时间序列变化指示了该湖泊早期发生洪水, 随着 $\delta^{18}O$ 值增加了 4‰, 指示了后期湖泊的蒸发量增加。同时, 由于没有“储库效应”, 保存在沉积物中的耳石也是测定放射性碳的良好材料。因此, 保存在沉积物中的化石耳石既是确定沉积年代的理想材料之一, 又是研究古气候环境的良好载体[37]。

4.2　耳石的 $\delta^{18}O$ 值与鱼类繁殖群体关系解析

青海湖裸鲤作为中国国家二级保护动物, 青海省自 1982 年开始实施封湖育鱼, 覆盖青海湖及所有入湖河流。当下正处于为期 10 年的第六次封湖期, 截至 2030 年 12 月 31 日。同时, 青海湖周边先后建立了原种保种基地和人工增殖放流站, 自 2002 年以来坚持开展人工繁殖放流工作, 累计向沙柳河和泉吉河放流裸鲤大规格鱼种 1.05 亿尾。因此, 青海湖中应该存在自然繁殖和人工繁殖两个群体, 仅仅通过耳石的 $\delta^{18}O$ 值难以区分该两大群体。

为了更加准确地识别鱼类种群、全面了解鱼类生活史, 很多学者将耳石的 $\delta^{18}O$ 值和 $\delta^{13}C$ 值结合分析, 因为耳石中 $\delta^{13}C$ 值的变化能够记录鱼类的成熟度和食物链的变化。例如, Gao 等[38] 分析了华盛顿州皮基湾太平洋鲱鱼的耳石的核心和第 2 年夏季季轮的 $\delta^{18}O$ 和 $\delta^{13}C$ 值, 认为皮基湾鲱鱼可能属两个鱼群; Ashford 等[39] 对位于巴塔哥尼亚大陆架和南佐治亚州的巴塔哥尼亚的美露鳕耳石进行了 $\delta^{13}C$ 和 $\delta^{18}O$ 值测试, 发现了 $\delta^{18}O$ 值反映了鱼生活的周围环境中 $\delta^{18}O$ 值和海水的温度, 能够对种群的来源进行辨别, 代谢碳来源与海水溶解无机碳(DIC)的 $\delta^{13}C$ 值影响着耳石 $\delta^{13}C$ 值。姜涛等[40] 利用稳定同位素质谱分析的技术, 第一次对长江口刀鲚的幼鱼耳石的碳、氧稳定同位素进行了初步的研究, 其中 $\delta^{18}O$ 指示了两组刀鲚起源的水体温度差异显著; 而 $\delta^{13}C$ 指示了饵料成分的差异。何勇凤等[25] 用养殖不同年龄组的四川裂腹鱼作为研究对象, 探讨稳定同位素与环境之间的关系。结果表明: 大于 1 龄的四川裂腹鱼耳石的质量与 $\delta^{13}C$ 和 $\delta^{18}O$ 值都没有显著的关系, 但在星耳石和微耳石之间却存在着明显的区别; 不同年龄的四川裂腹鱼微耳石 $\delta^{13}C$ 和 $\delta^{18}O$ 存在显著差异。耳石的 $\delta^{18}O$ 和 $\delta^{13}C$ 关联分析能够有效地对不同养殖年龄的四川裂腹鱼的群体进行区分, 因此, 可以作为一种对淡水鱼类养殖群体识别的方法。

5　结论

本文用 SHRIMP Ⅱ离子探针对青海湖裸鲤微耳石中的 $\delta^{18}O$ 测定分析, 研究表明所有裸鲤耳石的 $\delta^{18}O$ 值都呈现出成长带较低和间歇带较高的差异, 且能够记录裸鲤的洄游行为。核心区域差异性的 $\delta^{18}O$ 值则反映了裸鲤的产卵地和水温状况, 不同产卵场拥有差异性的水温、水流、底质等环境参数, 因此呈现出 3 种繁殖群体类型, 即有的裸鲤在水温较低的河口产卵孵化, 有的在水温较高的河流上游产卵孵化, 有的固定在河流生长繁殖。这种多样化的生活习性也是裸鲤应对不利生存环境进化而来的结果。

耳石的 $\delta^{18}O$ 稳定同位素成分研究在渔业和海洋环境科学方面有着广泛的应用前景。本文初步探讨了青海湖裸鲤微耳石中 $\delta^{18}O$ 值与洄游和产卵行为

的关系,这一结果为进一步揭示青海湖裸鲤的繁殖特性、洄游习性、生活史提供了有效信息。伴随着中国矿物微区原位分析与同位素地球化学研究的持续发展,青海湖裸鲤耳石的微区同位素分析和探索将更加深入。由于耳石化石能够保存在湖泊或海洋的沉积物中,也是推测古气候变化的良好载体,所以在青海湖沉积物中寻找裸鲤耳石化石工作也在持续进行。为了获取精准的青海湖水温与裸鲤耳石 $\delta^{18}O$ 值之间的响应关系,下一步将开展青海湖裸鲤的实验室温度、饲养水平控制实验,确定温度与耳石 $\delta^{18}O$ 值、饲养水平与耳石 $\delta^{13}C$ 之间的响应关系。

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Analyzing the Reproductive Characteristics of the Naked Carp *Gymnocypris Przewalskii* (Kessler) Based on the Oxygen Isotopes of Otolith Core Using SHRIMP

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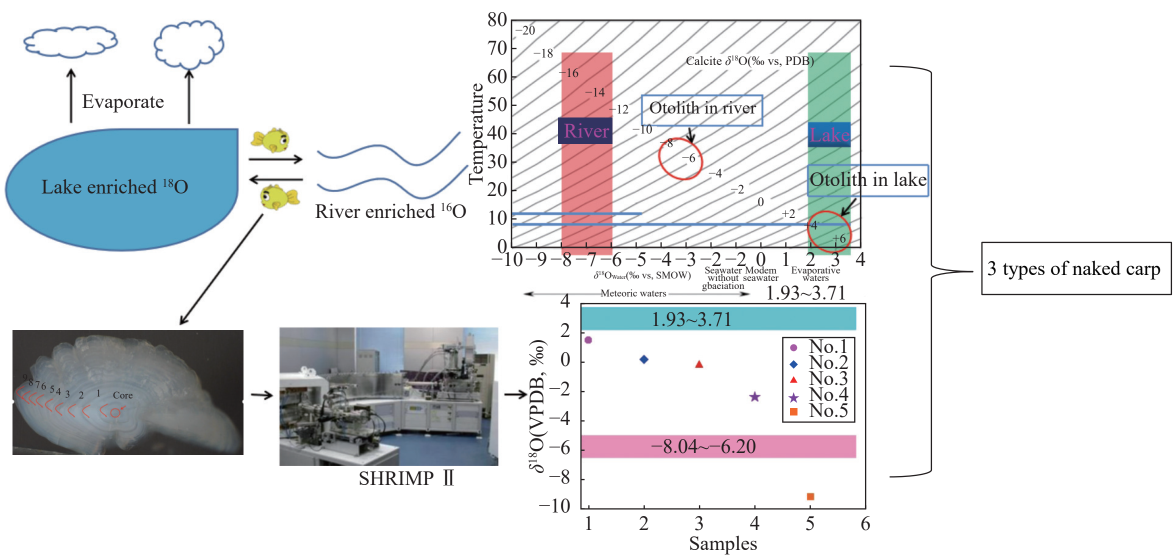
HIGHLIGHTS

(1) *In-situ* $\delta^{18}O$ composition of otolith was determined by SHRIMP Ⅱ ion probe, thus realizing the high-resolution microsample collection and analysis of otolith.

(2) The $\delta^{18}O$ composition of the otolith core reveals that there are three types of breeding population in naked carp.

(3) Based on the significant correlation between the otolith $\delta^{18}O$ and water temperature, the incubation temperature history of the naked carp was traced.



ABSTRACT

BACKGROUND: It is of great significance to study the breeding environment of the naked carp in Qinghai Lake for the conservation and restoration of natural habitat. The naked carp is the only economic species in Qinghai Lake, colloquially called "Huang Yu", and plays a core role in the lake ecosystem. As an anadromous species, the naked cap migrates between Qinghai Lake and major rivers to spawn such as the Buha, Shaliu, Quanji, Heima, and Hargai rivers from April to August every year. Due to intensive fishing, arid climate, and the lack of spawning rivers, the amount of the naked carp's resources has dropped sharply, from 28000 tons in the 1960s to 2263 tons in the 1990s. In view of its important ecological status, it is urgent to restore and protect the resources of naked carp in Qinghai Lake. Since 1982, Qinghai Province has forbidden fishing to restore and protect the fish resources. At the same time, it has established fish conservation bases and artificial breeding and releasing stations around Qinghai Lake. By 2022, the fish resources of the naked carp had increased to 108500 tons, 42 times as many as in the early stages of protection. The breeding environment is the key factor for the continuation of the fish population, and different fish require different parameters of the spawning ground, such as river, light, sediment and water temperature. It is also very important to obtain the reproductive environmental parameters and determine the optimal spawning ground for the protection and expansion of the fish resources. At present, only Zhou et al.[3] have discussed the environmental requirements of natural reproduction of the naked carp by indoor artificial simulation. Therefore, it is of great significance to study the breeding environment of the naked carp for the conservation and restoration of natural habitat.

Otolith δ^{18}O provides a method for fish population identification, migration and environment exploration of spawning ground. Otolith, a calcium carbonate mineral in bony fish, has both auditory and balancing functions and is known as the "recorder" of time and water environment. Microchemical analysis of otolith can reveal the life history and water environment changes of fish. Especially, the core area of otolith is formed at the early stage of incubation, and its microchemical composition can reflect the hydrochemical characteristics of the habitat and solve the problem of fish identification. The otolith δ^{18}O is closely related to water temperature. Under normal salinity conditions, the δ^{18}O change of 1‰ corresponds to the seawater temperature change of about 5℃. For example, the higher otolith δ^{18}O values of Pacific bluefin tuna, which migrated across the Pacific from the Western Pacific (WPO) to the Eastern Pacific (EPO), reflected the cold-water temperatures encountered during migration. Similarly, the lower δ^{18}O value in otolith core of *Oncorhynchus keta*, which migrated between freshwater and marine environments, indicated that Chinese salmon were also anadrotic spawning fish, returned to the ocean after hatching. Yuan et al.[12] analyzed the δ^{13}C and δ^{18}O values in the otolith of small yellow croaker in the Yellow Sea and the Bohai Sea, the population of southern Yellow Sea was subdivided into offshore and coastal populations for the first time, and no station crossing between the two populations was recorded. The overall successful rate of discrimination was recorded to be 82.6% by cluster analysis. Tatsuya et al.[13] conducted otolith δ^{18}O and microstructure analyses to investigate nursery habitat temperatures and early life growth rates. In conclusion, the otolith δ^{18}O provides a method for fish population identification, migration, and environment exploration of spawning ground. In this study, the SHRIMP Ⅱ ion probe was used to determine the otolith δ^{18}O of the naked carp in Qinghai Lake. Combined with the hydrochemical composition of lake water and river water in Qinghai Lake, the optimal migration and spawning sites and environmental conditions of the naked carp were investigated to provide effective information for further revealing its life history.

RESULTS: The SHRIMP Ⅱ ion probe was used to determine the *in-situ* δ^{18}O composition of the otolith microregion of five naked carp in Qinghai Lake. The *in-situ* oxygen isotope analysis of otolith was performed by SHRIMP Ⅱ ion probe at the School of Earth Sciences, Australian National University. Sectioned otoliths were

prepared for SHRIMP analysis by casting them in epoxy resin, with NBS 18 and 19 reference calcites, to form a 35 mm diameter mount. After being documented by optical photomicroscopy, the samples were coated with high purity Al and transferred to the ANU SHRIMP Ⅱ for analysis using procedures based on those described in detail by Long et al.[21-22]. In brief, the SHRIMP Ⅱ was operated in multi-collector, negative ion mode. A 15kV, ~3nA Cs^+ primary ion beam was focused to a 25mm diameter spot on the Al-coated target, producing 200−250pA of secondary $^{16}O^-$. $^{16}O^-$ and $^{18}O^-$ were measured simultaneously on Faraday cups using Keithley 642 electrometers. Each analysis consisted of a pre-burn of about 3 min to allow the secondary ion isotopic composition to stabilise, followed by 10 or 14 times (10s/time) estimates of the $^{18}O/^{16}O$ ratio. The accuracy was 0.1‰−0.2‰, and the standard deviation was about 0.3‰. The reference material was measured once every 5 samples completed during analysis. The spot starting position of the ion probe was the core of the otolith, along the longest growth axis to the edge. The spot diameter of the ion probe was about 25μm, and the spot depth was about 2−3μm. The spot test was shown in Fig.E.1A.

(2) The $\delta^{18}O$ composition along the growth axis of the otolith. The $\delta^{18}O$ ratios along the growth axis of 5 naked carp otoliths were shown in Fig.E.1B. The mean values of otolith $\delta^{18}O$ from No.1 to No.5 were 1.29‰, 1.79‰, 0.99‰, 0.93‰, −7.41‰ respectively, and their interval range were −4.88‰−3.46‰, −0.28‰−3.91‰, −1.43‰−2.94‰, −1.81‰−3.35‰, −9.36‰−5.21‰, respectively. The otolith $\delta^{18}O$ in individual juvenile plaice ranged from 0.2‰ to 1.9‰, when they were reared at two temperatures (11℃ and 17℃), which indicated that the otolith $\delta^{18}O$ ratios were significantly affected by water temperature, but not by feeding level, and there were no significant synergistic effects. The $\delta^{18}O$ ratios of plaice are close to the higher $\delta^{18}O$ ratios of samples 1 to 4 in this study, which is the result of the fact that naked carp lived in the lake with high salinity and low water temperature for part of the time. The *Schizothorax kozlovi* and the naked carp belong to the subfamily *Schizostomus*. The average otolith $\delta^{18}O$ ratios of the *Schizothorax kozlovi* cultured in fresh water is −9.4‰, which is very close to the $\delta^{18}O$ ratios of the naked carp No.5, indicating that sample No.5 has been living in fresh water. The gray bands are the incremental zones of otolith, and the interval zones between the incremental zones are discontinuous zones. The $\delta^{18}O$ ratios of the naked carp in samples 1 to 5 show a trend of lower incremental zones and higher discontinuous zones, because the $\delta^{18}O$ ratios of otolith is inversely correlated with water temperature. The incremental zones of otolith are formed in spring and summer, while the discontinuous zones in autumn and winter, and the water temperature in spring and summer is significantly higher than that in autumn and winter. The otolith $\delta^{18}O$ ratios of the five samples were analyzed by one-way variance analysis. The $\delta^{18}O$ ratios of the No.1, No.3 and No.4 naked carp were not significantly different (P>0.05), while the $\delta^{18}O$ ratios of the No.2 and No.5 naked carp were significantly different from the other three samples (P<0.05). The $\delta^{18}O$ ratios of Qinghai Lake ranged from 1.93‰ to 3.71‰, with an average value of 2.43‰, and that of surrounding rivers ranged from −8.04‰ to −6.20‰. The average $\delta^{18}O$ ratios of Buha River, Shaliu River, Quanji River and Heima River were −6.15‰, −7.21‰, −5.98‰, and −7.31‰, respectively. This is because the $\delta^{18}O$ ratios of different types of water bodies vary greatly, and the $\delta^{18}O$ ratios of lake water are obviously higher than that of surface river water due to strong evaporation. Comparing the $\delta^{18}O$ ratios of lake water and river water with that of 5 samples, it can be seen that the $\delta^{18}O$ signals of lake water and river water are recorded in the otolith of No.1-No.4, indicating that they migrated between lake water and river water, however, the $\delta^{18}O$ signal of river water was only recorded in the otolith of No.5, which indicated that the No.5 naked carp had been living in river water, that is, “sitting water fish”.

(3) Composition of $\delta^{18}O$ in otolith cores of the naked carp. The average $\delta^{18}O$ ratios of otoliths from No.1 to No.5 were 1.51‰, 0.20‰, −0.22‰, −2.38‰, and −9.14‰, respectively, and their interval range were 1.43‰−1.59‰, 0.28‰−0.7‰, 1.02‰−0.64‰, 4.88‰−0.74‰, 9.36‰−8.96‰. The average $\delta^{18}O$ ratios of otolith cores were compared with the $\delta^{18}O$ composition of lakes and rivers, as shown in Fig.E.1C. Since the core area of

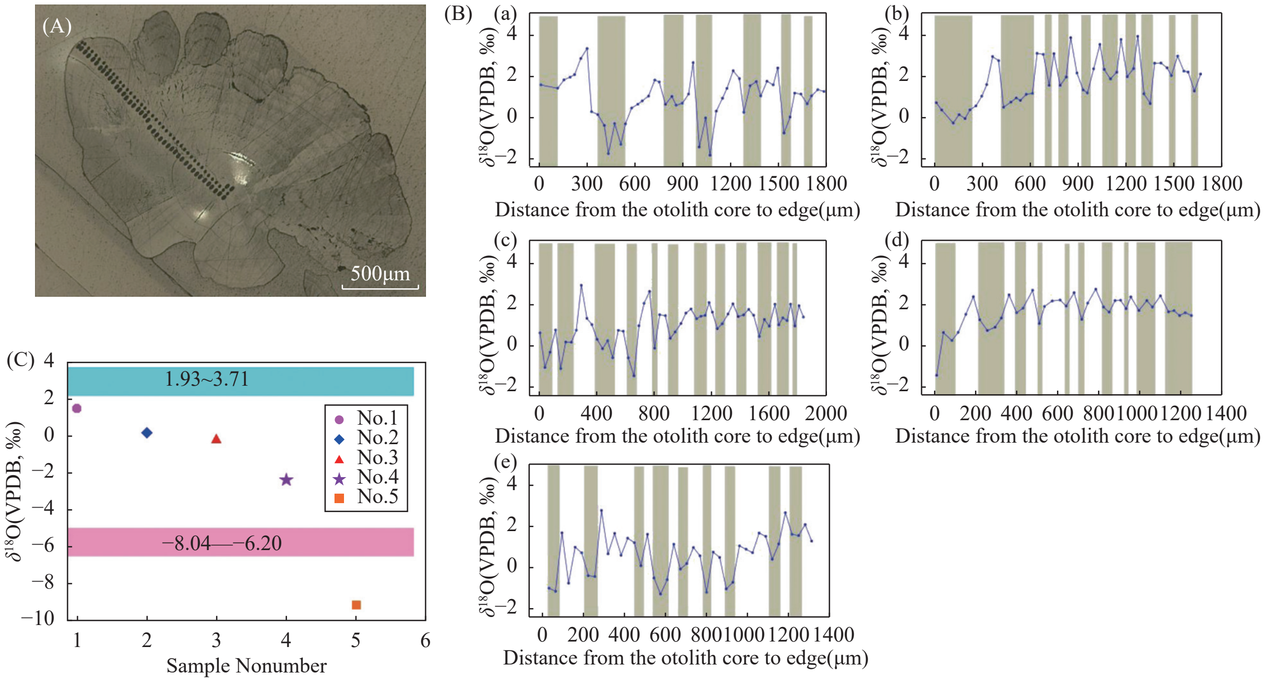


Fig. E1　Sample points, temporal changes in δ^{18}O, and distribution graph of δ^{18}O composition of the naked carp otolith core, lake water and major rivers. A—Sample points of otolith in naked carp by ion probe. B—Composition of δ^{18}O from core to edge of otolith in naked carp. (a)-(e) in the figure correspond to otolith samples 1-5, respectively. The gray band is the incremental band in the otolith. C—Distribution graph of δ^{18}O composition of the naked carp otolith core, lake water and major rivers. The δ^{18}O values of the otolith core in the figure are the average values of the core area, with blue bands indicating the δ^{18}O range of the lake and pink bands indicating the δ^{18}O range of the river.

otolith was formed in the early incubation period of naked carp, the water environment at birth was recorded. From the distribution, the δ^{18}O ratios of the core area of naked carp No.1-No.3 were closer to the lake water, which indicated that their spawning place was near the estuary. The water body was mixed with lake and river water, and having lower water temperature, which indicated the migration and spawning may have started in late May. The δ^{18}O ratios of the core area of the No.4 naked carp was closer to the river water, which indicated that the spawning area was far away from the lake and the water temperature was higher, while the No.5 naked carp lived in the river all the time and the water temperature was higher when spawning. In conclusion, the habits of the naked carp are not fixed. Some breed in estuaries, some in upstream rivers, and some migrate between rivers and lakes to spawn, and a small part of the naked carp breed in rivers, which may be related to the local rivers' cutout in autumn and winter. Some fish that migrate to the upper reaches of rivers to spawn have stayed in rivers for breeding due to the cutout of rivers. Similarly, the otolith cores of Pacific herring born in the Strait of Georgia have lower δ^{18}O ratios (−8.2‰ to −2.0‰), while the otolith cores of the southern Puget Sound samples have higher δ^{18}O ratios (−3.9‰ to −0.9‰).

DISSCUSION: (1) The relationship between δ^{18}O ratios of otolith and water temperature. The otolith of the naked carp can record not only the age, but also the water environment and the life history of the fish. The otolith δ^{18}O ratios of fish is determined by the δ^{18}O ratios of water body and the water temperature. The δ^{18}O ratios of the water body are related to the ratio of precipitation to evaporation, runoff and precipitation, while there is an inverse correlation between temperature and otolith δ^{18}O ratios. The living environment of fish can be inferred from the δ^{18}O ratios value on the otolith of fish, and the isotopic balance between the surrounding water environment and aragonite minerals is reached or almost reached, therefore, the fractionation of ^{16}O/^{18}O isotopes is closely related to the temperature of the water body, generally showing a negative correlation. For marine fish, the variation of $\delta^{18}O$ ratios at 1‰ corresponds to the variation of sea temperature at about 5℃ under normal salinity conditions. The relationship between temperature variation and otolith $\delta^{18}O$ ratios of freshwater fish at 1‰ has not been established.

(2) Experimental research about temperature control on otolith $\delta^{18}O$ ratios. Water temperature, water flow, water depth, riverbed quality and light are the most important environmental parameters in fish breeding. In general, the reproductive activities of fish are not determined by a single environmental factor, and their specific reproductive needs are often multiple environmental factors. When the water temperature of the freshwater tributaries around Qinghai Lake reaches the threshold of 6−16℃, the naked carp will start to migrate, on the one hand, due to the different composition of $\delta^{18}O$ ratios in different spawning sites, and on the other hand, due to the different spawning time, the difference of water temperature is recorded in the otolith $\delta^{18}O$.The contribution of the $\delta^{18}O$ composition and the change of water temperature to the $\delta^{18}O$ ratios in the otolith of the naked carp cannot be quantified, and needs to be further verified by the artificial control experiment. For example, Nakamura et al.[31] cultured mackerel larvae at six different temperatures (16.3℃, 17.6℃, 18.3℃, 20.0℃, 24.0℃ and 26.5 ℃) and determined the $\delta^{18}O$ ratios, the linear relationship between the water temperature and the otolith $\delta^{18}O$ of mackerel was determined. Willmes et al.[32] reared male oversea fish for 360 days at different salinity (8.75‰, 5.28‰ and 4.06‰) and water temperature (16.4℃, 16.7℃, 18.7℃ and 20.5℃), and then reconstructed the thermal life history of the fish. Wang et al.[33] evaluated whether cooking behavior would lead to further isotopic fractionation of carbonate in otolith and showed that the otolith $\delta^{18}O$ ratios were highly consistent between cooked and uncooked, which indicated the cooking process has no or little effect on the otolith isotope value.

(3) Fossil otolith is a good carrier to study the paleoclimate environment. Otoliths are well preserved in sediment, so the fossil otoliths are often used in paleoclimate, palaeoecology and palaeogeography. For example, Wurster et al.[34] analyzed high-resolution $\delta^{18}O$ ratios of *Aplodinotus grunniens* from the Easterman archeological site to infer climate change in the eastern continental United States during the Holocene. Andrus et al.[35] used the otolith $\delta^{18}O$ ratios of a well-preserved Peruvian catfish fossil to calculate the sea surface temperature of the Peruvian Sea in the Middle Holocene. Surge et al.[36] analyzed the $\delta^{18}O$ ratios of modern and circa 2nd−3rd century AD fossil otoliths of the Mexican sea catfish, and the recovered winter temperatures were very similar to those of modern times. Long et al.[21] analysed the $\delta^{18}O$ ratios of otolith in the sediment of Mungo Lake, Australia, and the variation of the $\delta^{18}O$ ratios indicates an early flood in the lake, a subsequent increase in the $\delta^{18}O$ by 4‰ indicating increasing evaporation of the lake. At the same time, because there is no "Reservoir effect", the otolith preserved in the sediment is also a good material to measure radioactive carbon. Therefore, fossil otoliths preserved in sediments are not only one of the ideal materials to determine the age of sediments, but also a good carrier to study the paleoclimate environment.

(4) Relationship between $\delta^{18}O$ ratios of otolith and fish breeding population. The naked carp in Qinghai Lake is one of the second-class national protected animals in China. Since 1982, Qinghai Province has implemented the closure of the lake to breed fish, covering Qinghai Lake and all the rivers entering the lake. Currently, the sixth closure period in 10 years will end on December 31, 2030. At the same time, the original species preservation bases and artificial breeding and releasing stations have been established around Qinghai Lake. Since 2002, artificial breeding and releasing work has been carried out, and 105 million large-size naked carp fry have been released into the Shaliu and Quanji rivers. Therefore, there should be two populations of natural reproduction and artificial reproduction in Qinghai Lake, and it is difficult to distinguish the two populations only by the otolith $\delta^{18}O$ ratios.

(5) In order to identify fish populations more accurately and understand fish life history, many scholars combine the $\delta^{18}O$ and $\delta^{13}C$ ratios of otolith for analysis, because the change of otolith $\delta^{13}C$ ratios can record the maturity of fish and the change in the food chain. For example, Gao et al.[38] analyzed the $\delta^{18}O$ and $\delta^{13}C$

ratios of the otolith core and the second summer season round of Pacific herring in Pikew Bay, Washington, and concluded that there are two types of herring. Ashford et al.[39] tested the $\delta^{18}O$ and $\delta^{13}C$ ratios of cod otolith located on the Patagonia continental shelf and Patagonia in southern Georgia, and found that the $\delta^{18}O$ ratios reflected the sea water temperature in the surrounding environment where the fish lived, so that the different population could be identified. The source of metabolic carbon and the $\delta^{13}C$ ratios of dissolved inorganic carbon (DIC) in seawater affect the $\delta^{13}C$ ratios of otolith. For the first time, Jiang Tao et al.[40] analyzed otolith stable isotope ratios of $\delta^{18}O$ and $\delta^{13}C$ using an isotope ratio mass spectrometry on *Coilia nasus* juveniles collected from the Changjiang River Estuary. The different environments with different water temperature and food organism composition might be experienced by the two groups of *C. nasus* fish. He Yongfeng et al.[25] took *Sichuan schizostomus* of different age groups as the research object to explore the relationship between stable isotopes and environment. The results showed that $\delta^{18}O$ and $\delta^{13}C$ ratios of *Schizothorax kozlovi* at age 1t were not significantly correlated with otolith mass, but both were significantly different between lapillus and asteriscus. There was no significant difference of lapillus $\delta^{18}O$ and $\delta^{13}C$ between the sexes, but with significant difference among different ages. The correlation of $\delta^{18}O$ with $\delta^{13}C$ was an effective method in identification of different culture stocks of *S. kozlovi*, which indicates that the isotopic signatures of otolith could be used as a method to identify freshwater fish stocks.

KEY WORDS: Qinghai Lake naked carp; spawning grounds; otolith; oxygen isotope; SHRIMP; water temperature

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